University of Alberta

Cycling nitrogen for productivity in agroforestry: nitrogen, lignin and polyphenol controls on mineralization

by

Michael Jonathan Cody

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

in

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University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Cycling N for productivity in Agroforestry; nitrogen, lignin and polyphenol controls on mineralization* submitted by Michael Jonathan Cody in partial fulfillment of the requirements for the degree of Master of Science in Agroforestry.

Dr. W.B. McGill - Supervisor

Dr. J.C. Alegre

Dr. D.S. Gill

Dr. D.C.L. Kass

Dr. R.L. Rothwell

Date: 21 Jan/99

For my wife Gillian

Abstract:

Soil productivity in the Humid Tropics depends on conservation and recycling of nutrients within the soil-plant system. In agroforestry systems, efficient use of organic fertilizers requires that nutrient release from them be timed for maximum uptake by crops and minimal leaching or gaseous loss. Chemical constituents such as nitrogen (N), lignin (L), and polyphenols (PP) are controls on rates of N release. Patterns of N release from the prunings of two tree species and one herbaceous legume were investigated in relation to the chemical characteristics of the materials. Also, N distribution in the soil following slash and burn disturbance was documented. Mixing of pruning types reduced the predictability of N release; interaction between mixed components was consistent with the action of soluble polyphenols. Overall, L+PP:N ratio was the best chemical index for prediction of N release. Slash and burn disturbance resulted in a mineral N flush in the first metre of soil; some of this N was leached below one metre.

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1. Introduction

Manipulation of nutrient cycles is basic to modern agrarian production. Tillage and mineral fertilization are examples of technologies which improve yields by controlling nutrient supplies to crops. Such technologies perturb element cycles and can result in social costs: they are energy intensive and contribute to air and water pollution when elements are transported out of soils. In most stable, non-managed soil-plant systems, nutrient additions and losses are small while nutrient cycling within the system is vigorous. In large areas of the Humid Tropics the general characteristics of high temperatures, heavy precipitation and highly weathered soils result in nutrient losses from the soil-plant systems under agrarian production. The potential for nutrient loss from soil-plant systems, combined with poor economies and increasing population pressure, implies that productivity in the Humid Tropics depends upon conservation of nutrients within soil plant systems. I argue that production systems which incorporate vigorous nutrient re-cycling within the soil-plant system have the greatest value to people.

The traditional farming system of the humid tropics, shifting cultivation, does not conserve nutrients within the soil plant system, but depends on adequate fallow periods in the order of decades during which soil fertility can be restored. Shifting cultivation is both productive and sustainable provided fallow periods are sufficient, but as human population increases fallow periods have tended to decline, especially near settlements and access routes into humid forests. Shortened fallow periods are believed to cause soil nutrient depletion (Sanchez and Buol 1974). As well as concerns about soil degradation, the cycle of population increase, shortened fallow periods, and continued agrarian encroachment on tropical forests is causing concerns about forest and bio-diversity loss (Bowyer 1997).

These environmental problems are exacerbated by an expanding human population and increasing demand for food. The world already has a serious hunger problem, to the

extent that about one fifth of humans are hungry each day (Daily et al. 1998) and 730 million have so little food that they are functionally impaired (Streeten 1995). The majority of people subject to chronic undernourishment live in the tropics of South Asia, Sub-Saharan Africa, and Latin America (IIASA 1987). Poverty is closely linked to hunger, and the problem is partially a result of the loss of economic entitlement for food (Sen 1983). Although the worlds total production of food may be sufficient it is not distributed equally and there are regional production shortfalls. The world capacity to increase food production is in question, and even optimistic projections suggest global production shortage in the next century (Kendall and Pimental 1994).

The sustainability of production systems has recently come into question as the social-environmental costs of agricultural production become apparent (Daily et al. 1998). This is especially conspicuous in soils, where the longevity of economically viable production may be approximately equal to the period of organic matter loss during cropping (Tiessen et al. 1994). In the tropics, organic matter loss can be particularly fast (Sanchez 1976; Tiessen et al. 1994), because warm temperatures and adequate moisture provide optimal conditions for mineralization of nutrients. Mineralized nutrients are lost if crop nutrient uptake is not synchronized with their release, and of sufficient magnitude to incorporate them into plant biomass. Nitrogen is especially susceptible to loss from humid tropical soil-plant systems because year around warm temperatures and heavy precipitation favor leaching and gaseous losses, and N is susceptible to both.

High yielding, continuous crop production on highly leached, acid infertile tropical soils is possible. Sanchez et al. (1982) demonstrated such yields at Yurimaguas, Peru using adequate inputs of mineral fertilizers and lime (Sanchez et al. 1982). They suggest that increased global requirement for food can be partially met by high-input production on large areas of level, well drained Ultisols and Oxisols in the Amazon Basin. These soils have chemical production constraints but favorable physical properties, and make up 43% of the Amazon Basin. Although input and yield quantities under this continuous

production technology were not unlike those of industrialized agricultural production, high-input production has yet to be widely adopted in the region. Economies of developing countries may lack the stability and infrastructure to support high input systems. Further, high-input systems are coming under increasing scrutiny as genetic diversity in crops declines, inputs become pollutants, and production is linked to climate change (Daily et al. 1998). N loss to ground water from high-input systems is a direct economic cost to farmers, threatens water quality, and is of concern both in industrial and developing nations (Webster et al. 1993; Babbar and Zak 1995). Production of N fertilizer is extremely energy intensive, and unless offset by sufficient increase in plant biomass production, contributes to increasing atmospheric CO_2 concentrations and possibly accelerated climate change.

Conservation and recycling of nutrients within the soil plant system is reflected in the concept of synchrony. The objective of synchrony is nutrient release coupled with temporal and spatial plant nutrient demand (Myers et al. 1994). Released nutrients are susceptible to losses from the system, but if the timing, location and rates of release are synchronized with plant nutrient uptake, the potential for losses is minimized (Figure 1.1). Synchronous nutrient transformations can be approached by managing nutrient release during decomposition, crop nutrient uptake, or both. Nutrient release may be managed with an understanding of the bio-physical controls on decomposition and knowledge of the site- and species-specific rates of nutrient release. Some controls on decomposition may be manipulated, such as the chemical characteristics of applied organic fertilizers. Knowledge of nutrient release rates may allow timing of nutrient release to coincide with crop demands. Characteristics of crop nutrient uptake vary with species, and timing of cropping season; these characteristics can be matched to nutrient availability for synchrony.

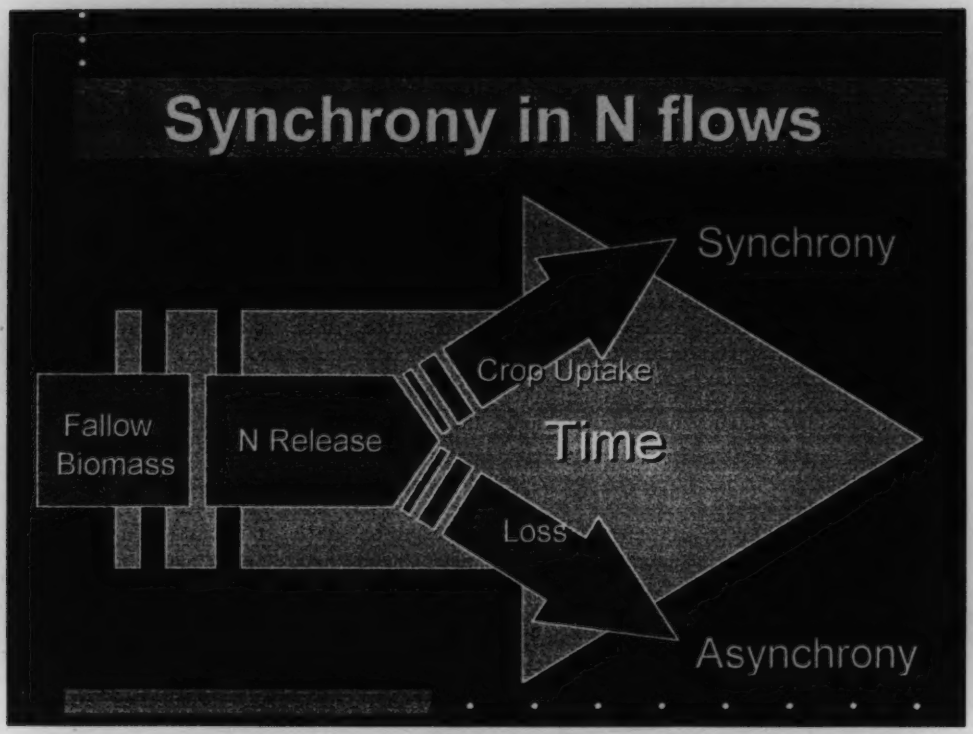

Figure 1.1 A schematic depiction of synchrony.

A viable means to conserving nutrients within soil plant systems and reducing mineral N requirements may be the inclusion of trees into production systems (agroforestry) and use of organic fertilizers produced in these systems. Trees may add N to soil plant systems through biological N fixation and increase recycling of nutrients within the soil-plant system, imitating the conservative dynamics apparent in forests.

Agroforestry systems do not guarantee synchrony however, because organic fertilizers are also susceptible to loss if their mineralization is not followed by crop uptake. Synchronization of nutrient flows in agroforestry requires that a predictive understanding of litter decay rates and the mobility, root availability, and uptake of released nutrients be developed. Decomposition of litter and prunings is partially controlled by the chemical

characteristics of the decaying materials. Nitrogen, lignin and polyphenols are among the important chemical constituents which control decomposition. Predictive capabilities must be linked to knowledge of tree species characteristics such as rooting habit and litter chemistry.

Agroforestry systems may be classified according to their structure, which is defined as the arrangement of system components in time and space (Nair 1989). Alley cropping systems include trees simultaneously with crops, with components arranged in rows along side each other. Improved fallow agroforestry systems separate the tree and crop components in time. When crop yields are analyzed in alley cropping, the costs of tree/crop competition are sometimes greater than the soil fertility benefits imparted by trees (N additions and vigorous nutrient cycling) (Sanchez 1995). In response to this competition problem, and limited adoption by farmers, some of the focus of agroforestry research has shifted from alley cropping to improved fallows, which avoid tree/crop competition. Improved fallows, however, rely on efficient transfer of nutrients accumulated during the fallow to subsequent crops. Synchrony in nutrient transformations is thus conceptually crucial to the success of improved fallows.

The primary objectives of this work were to study N release from agroforestry prunings in relation to chemical characteristics of the prunings, and to study N distribution in the soil following slash and burn disturbance of an improved fallow agroforestry system. Chapter 2 documents an experiment in which prunings of contrasting N, lignin, and polyphenol contents were applied in an improved fallow agroforestry system at Yurimaguas, Peru. Their chemical characteristics are related to N release as measured by plate lysimeters and porous cup suction lysimeters. Slash and burn was an important control on nutrient cycling in the improved fallow system, so mineral N distribution in the mineral soil following this disturbance is characterized in Chapter 3. Methods of sampling for soil N, and relationships between sampling techniques, soil N status and crop yield are investigated.

Chapter 4 documents an experiment conducted at CATIE in Turrialba, Costa Rica, where N release from differing prunings and was investigated using a Costa Rican Andisol and the Peruvian Ultisol (used in experiments at Yurimaguas) in a leaching column incubation design under greenhouse conditions. In Chapter 4 the relationships between chemical characteristics of prunings, soil type, and the patterns of N release are investigated; mathematical mineralization models are used to help characterize N release patterns. In Chapter 5 (synthesis), the knowledge base required to manage for synchrony, and the value of synchrony to terrestrial production systems is discussed.

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2. N mineralization from prunings within an improved fallow agroforestry system

2.1 Introduction

An ability to understand and thereby manipulate decomposition and nutrient release in agroforestry systems is critical because conservative nutrient cycles are a tenet of agroforestry. Conservative nutrient cycles are synchronous: nutrient mineralization or plant availability is coupled with plant uptake so that losses from the soil-plant system are avoided. Decomposition of organic matter may last for centuries, and rates of nutrient release vary during decomposition. Thus achieving synchrony when using a nutrient rich mulch may be focused on the current season, building soil organic matter, or both.

N mineralization from prunings and litters of agroforestry species was studied by many authors in laboratory experiments, green house settings and in some cases with the use of litter bags in the field. Initial N content, lignin content (L), polyphenol content (PP) and protein binding capacity (PBC) were examined as predictors of N mineralization. Lignin plus polyphenol:N ratio (L+PP:N) was commonly found to be among the best predictors of N mineralization (Fox et al. 1990; Constantinides and Fownes 1994; Handayanto et al. 1994 and 1997; Lehmann et al. 1995). Others found polyphenol:N (PP:N) ratio to be the best predictor (Palm and Sanchez 1991; Oglesby and Fownes 1992). Recent work by Handayanto et al. (1994 and 1997) elegantly connects the PBC imparted by soluble phenolics in prunings with N mineralization and also maize N uptake from prunings. Carbon to nitrogen ratio (C/N) is a critical control on N mineralization in many environments (Mellilo et al. 1982) , but agroforestry materials often have C/N ratios (20 or less), below the threshold where materials begin to immobilize N. Nevertheless, initial N content is closely connected with N mineralization from both non-legumes and legumes in some studies (Frankenburger and Abdelmagid 1985; Tian et al. 1992; Constantinides and Fownes 1994).

Chemical characteristics of substrates, such as polyphenol, lignin or nitrogen content are believed to be the most important control on decomposition rates in the tropics (Meentemeyer 1978) and has received the most attention, but it is possible that other controls such as climate or characteristics of the microbial and faunal communities are also important. Most investigation of litter and pruning quality in agroforestry systems employ laboratory or greenhouse incubations (Fox et al. 1990, Palm and Sanchez 1991, Tian et al. 1992, Oglesby and Fownes 1992, Constantinides and Fownes 1994, Handayanto et al. 1995 and 1997). In contrast, fewer field experiments are documented; Palm and Sanchez 1990, Montagnini et al. 1993, and Lehmann et al. 1995 all used the litter-bag technique. Many incubations use oven or air dried substrates but this may lower rates of decomposition dramatically (Taylor 1998). Ground substrates are often used in incubations; grinding homogenizes and increases surface area, possibly raising decomposition rates (Fyles and McGill 1986).

Although mixed litters are common in either agroforestry or natural systems, little investigation into N mineralization from mixes, and interactions between mix components, exists. Combining materials with differing chemical characteristics may allow management of the net rate of N mineralization from the mix to meet synchrony objectives (Myers et al. 1995; Handayanto et al. 1997). Various hypotheses about interaction among mix components are proposed. Swift (1987) suggests that a mix of high and low quality materials would initially resemble mineralization of the low quality component and later the high quality component, forming a sigmoidal mineralization curve. Palm (1995) notes that mixes tend to simulate the high quality component. Handayanto et al. (1997) suggests that interaction between mix components will only occur if there is sufficient quantity of soluble carbon (including phenolics) which can move between components. Mixing increases the diversity of microbial substrate and possibly also the diversity of the establishing microbial community (Blair 1990); community level controls on N mineralization may also be important (Janzen et al. 1995).

The goal of this experiment was to work in the initial phase of mineralization with different types of agroforestry prunings and attempt to predict and to manipulate nitrogen (N) mineralization in a field experiment. Three hypotheses were tested:

- First, that the initial chemical characteristic of either PP:N or L+PP:N would be significantly correlated with N flows *in-situ* an improved fallow agroforestry system.
- Second, that N mineralization from a mix of low and high L+PP:N prunings could be initially reduced below rates predicted by the mean of the components' individual mineralization rates. This hypothesis tests for interaction between mixed components at the beginning stages of decomposition through movement of soluble pruning constituents.
- Third, that prunings of high lignin and phenolic content will receive less faunal attack than materials low in these constituents, as tested by controlling faunal access to mulches with meshes of differing sizes.

2.2 Materials and Methods

Experimental Site:

The experimental site was located on the lands of the San Ramon experiment station of the Instituto de Investigacion Amazona Peruano (IIAP) and the International Centre for Research in Agroforestry (ICRAF) at Yurimaguas, Peru (76°05'W, 5°45'S, 180 m elevation). This region is characterized by a mean annual temperature of 26° C and 2200 mm of annual precipitation. Previous workers at the station have not noted a marked dry season: Palm and Sanchez (1990) note well distributed precipitation, with about 200 mm each month except for June, July and August which received about 100 mm, and Seubert et al. (1977) suggest that there is no pronounced dry season. In recent years, however, the June - August dry season was considerably more marked receiving well below 100 mm per month (Arevalo et al. 1998). In 1997 conditions remained very dry from July to early October (Figure2.1).

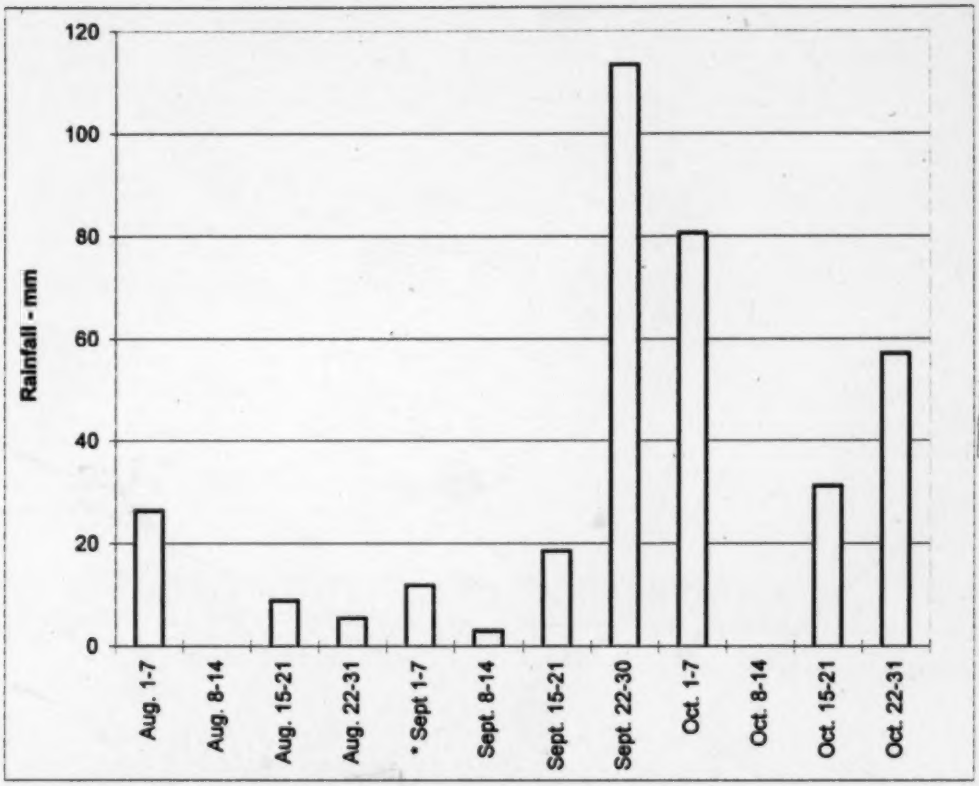

Figure 2.1 Weekly rainfall at Yurimaguas Experiment Station. * = pruning application (05 Sept).

Experiments were conducted within an improved fallow agroforestry system operated by ICRAF (See Appendix 5, photo #1). Fallow crops of the improved fallow system were established in October of 1994 on a nutrient depleted site recently abandoned by a local farmer. The treatments of the improved fallow experiment were established in 5 X 20 meter plots in a randomized complete block design with 4 blocks. The site is an upland terrace with soil classified as a fine-loamy, siliceous, isohyperthermic, Typic Paleudult (Tyler 1975) (See Appendix 5, photo #2). Organic C and extractable P dropped sharply, and aluminum saturation increased sharply, between the 0-5 and 5-15 cm layers sampled (Table 2.1).

Table 2.1 Soil Characteristics - Yurimaguas improved fallow Site

	Db	pH	Org. C (g/kg)	N (g/kg)	P (mg/kg)	CEC (cmol+/kg)	Al Satn. %
0-5	1.21	5.3	12.11	1.22	7.25	2.21	49.58
5-15	1.30	5.2	7.95	0.81	1.67	2.42	87.07
0-15 (combined)	1.27	5.2	9.34	0.95	4.46	2.32	74.57

Source: J.C. Alegre, ICRAF (unpublished data), sampled November 1995.

Plant Materials:

Three species from the improved fallow system were examined in this experiment: *Inga edulis* (common name Guava), *Centrosema macrocarpum* (common name *Centrosema*) and *Colubrina glandulosa* (common name Shaina). *Inga edulis* is a multi-purpose tree legume highly regarded by local farmers for its benefits as a soil-improver and for food and fuel-wood. *Centrosema macrocarpum* is a herbaceous legume exotic to the region which accumulates large amounts of N rich biomass. *Colubrina glandulosa* is not an N-fixing tree, but is very fast growing, producing valuable construction wood. In the improved fallow experiment, these species were grown in 5 treatments: each of the three species in pure stands plus combinations of *Centrosema* mixed with *Inga*, or *Colubrina*.

Sub-plots:

At the start of this experiment (September 1997) it was expected that there could be chemical and physical soil differences imparted by 3 years growth of the managed fallow vegetation. As well, it was expected that the N, lignin and polyphenol contents of the vegetation and litter might be characteristic of the species and the growing conditions of each treatment. Thus to study decomposition of the fallow vegetation, sub-plots ($1m^2$) were established within the improved fallow experiment and prunings were cut from and

returned to their specific treatments (for example prunings from *Centrosema macrocarpum* grown in mixture were returned to a sub-plot located in a plot of the same mix treatment). Sub-plots were established by cutting and removing all vegetation and litter, and cutting all roots to a depth of 25 on the perimeter of the 1 m^2 area (See Appendix 5, photo #3) Roots at the perimeter of the sub-plot were cut again after 1 month (02 October 1997). The mineral soil surface was disturbed as little as possible. 24 sub-plots were prepared, allowing for 12 experimental (4 treatments X 3 replicates) sub-plots and 12 control (no pruning application) sub-plots.

Suction Lysimeters:

On 29 August 1997, porous cup suction lysimeters (SLs) were installed in each of the sub-plots at plot centre. Before installation, porous cups were washed and soaked, first in 5% HCl for 24 hours, and later deionized water for 24 hours. Lysimeters were installed by excavating a hole slightly larger than the lysimeter diameter. A thick slurry, made from excavated soil and deionized water, was used to coat the porous cup and poured into the hole before insertion of the lysimeter. Lysimeters were inserted to a depth of 15 cm, dry soil was filled around the lysimeter tube and gently compacted until the instrument was firmly installed.

Prunings:

Prunings of *Inga* and *Colubrina*species were cut from plots on 02 September 1997 and *Centrosema* on 03 September. On 03 September all prunings were cut to pass through a 10 cm aperture mesh. Prunings of the tree species included only leaves and petioles while *Centrosema* prunings included leaves, petioles and herbaceous stems. Efforts were made to use pruning material as they would be realistically utilized by a farmer in an improved fallow system, consequently woody material was not included (wood is commonly utilized for fuel or construction material). On 04 September prunings were weighed to pre-calculated fresh masses equivalent to 1.2 kg m^{-2} dry material (12 Mg ha^{-1}). Subsamples were taken for analysis of pruning characteristics and to determine moisture

content change between time of cutting (02/03 September) and time of weighing (04) September. All materials were stored in large porous plastic bags during handling (02-05 September) and some heating of the materials was noticed, most notably in the *Centrosema* prunings. On 05 September 1997 prunings were applied to sub-plots for decomposition (See Appendix 5, photo #4).

Plate Lysimeters:

Plate lysimeters (PLs) were established in sub-plots on 05 and 08 September. PLs were prepared using disposable plastic plates (20 cm diameter) covered by nylon mesh fabrics. All materials were washed and soaked first in 5% HCl for 24 hours and then in deionized water for 24 hours. Similar to litter bags placed upon plastic plates, the PLs were constructed by stapling a fine (.35 mm aperture) bottom screen and either course (3.5 mm) or fine (.35 mm) top screen to the plastic plate; a small hole was cut at the plate centre for collection of leachate. On 04 September plate lysimeters were loaded with material at a rate of 4 Mg ha^{-1}; the PL design would not accommodate the rate of 12 Mg ha^{-1} at which prunings were applied to sub-plots. In the field, PLs were installed so that test tubes could be placed below the hole in the PL for collection of leachate (see photo in Appendix 5). 3 control PLs (without any added prunings) were also established.

Sampling Protocol and laboratory analysis:

We aimed to minimize sample storage time in SL porous cups or PL test tubes, and we analyzed samples as rapidly as possible. Samples were taken following moderate-heavy rainfall events (10 - 40 mm), once per week. Immediately following rain, test tubes containing PL samples were removed and carried to the lab for analysis. Up to 12 hours following an event, SLs were evacuated and samples recovered the following day. Each Monday clean test tubes were installed below PLs for sample collection. The sampling period began on September 8 and 17, 1997 and extended to October 22 and 23, 1997, for PLs and SLs respectively. Prior to analysis, PL samples were filtered with Whatman #42

paper to remove soil particles from the sample. Analysis for NH_4^+ and NO_3^- in water samples was completed colorimetrically by the indophenol blue and the salicylic acid methods respectively (Keeney and Nelson 1982). Polyphenols were extracted from oven-dried (60°C) pruning samples by immersion in 50% aqueous methanol at (78-80°C) for one hour. Polyphenols in the extract were measured colorimetrically using the Folin-Denis reagent against a tannic acid standard (Anderson and Ingram 1989). Lignin in oven-dried (60°C) pruning samples was measured by the acid detergent fibre method using permanganate oxidation step for lignin (Van Soest and Wine 1968). Total N in prunings was measured by semi-micro kjeldahl digestion followed by distillation and titration (Bremner and Mulvaney 1982). Total carbon was measured by dry combustion using an automated LECO C analyser (McKeague 1978).

Data conversions:

Water samples were analyzed in the laboratory for mineral N concentration. Mineral N concentration was used to calculate the quantity of N release during the rainfall event, and this quantity expressed as a percentage of the total N contained in the applied prunings. For PLs the volume of rain collected by the plate was multiplied by the sample concentration in the test tube. Note that test tubes used to collect PL samples were not large enough to contain the whole water volume collected by the plate. Once the receptacle test tube was full, excess sample simply over-flowed into the soil. The volume of rain collected by the plate was calculated as the product of the plate area and the amount of rain (Appendix 2).

The mineral N concentration sampled by the SL's was assumed to represent the mineral N concentration in the soil solution of the treatment. The mineral N concentration was multiplied by the volume of water in the soil layer sampled by the lysimeter yielding a quantity of soil solution mineral N per unit area (Appendix 3). The difference between soil solution mineral N in treated and control plots was considered to be N attributable to release from prunings. This quantity was then expressed as a percentage of the total N in

applied prunings. It was assumed that the porous-cup suction lysimeter sampled soil water held between zero tension (0 Mpa) and field capacity (1.5 Mpa).

Statistical Analysis:

Regressions among chemical characteristics of prunings, and between N mineralization and chemical characteristics were conducted using Microsoft Excel 7.0. SAS software was used for each of the following: PL treatment mean separations by analysis of variance and least significant difference. T-tests of the difference between PL mesh sizes. SL mean separations by analysis of covariance procedure (see Appendix 1) using SAS software.

2.3 Results

Pruning Characteristics:

Initial chemical characteristics of prunings are summarized in Table 2.2. The C:N ratio of the materials employed ranged from 13.0 (*Inga*) to 17.4 (*Colubrina*) and L+PP:N ranged from 10.8 (*Inga*) to 5.2 in (*Centrosema*). %PP and %L were very highly correlated with each other in these materials (Table 2.3).

Because this experiment was conducted within the larger improved fallow system, foliage for the Mix treatment was taken from *Inga* and *Centrosema* plants growing in mixture with each other. Some intra-species differences were noticed between prunings originating from mixed stands versus prunings originating from pure stands (Table 2.4). In *Inga*, %N and %PP were slightly higher, and %L slightly lower, when grown in mixed versus in pure stands. In *Centrosema*, the opposite trend was apparent: %N and %PP were lower, and %L higher, when grown in mixed versus pure stands. The Mix was not composed of equal proportions *Inga* and *Centrosema* because moisture contents changed during handling and actual dry masses applied were not perfectly (50/50) proportional; the actual mix applied was 39% *Inga* and 61% *Centrosema* on a dry mass basis. In Table 2.5 the pruning characteristics of the actual Mix are compared to 2 sets of hypothetical Mix

characteristics based on a) equal proportions of each component, and b) prunings from pure stands.

Table 2.2 Initial chemical characteristics of prunings from 3 species and one mix of species.

	%C*	% N *	% PP *	% L *	L+PP:N	C:N
Inga pure	46.49	3.58	3.78 (0.001)	34.8 (2.6)	10.8	13.0
Centrosema pure	46.27	3.29	1.46 (0.001)	15.6 (0.8)	5.2	14.1
Colubrina pure	48.32	2.78	3.02 (0.021)	30.2 (0.3)	12.0	17.4
Inga & Centrosema Mix	46.36	3.32	2.28	24.3	8.0	13.9

Lignin (L) and polyphenol (PP) analysis completed in duplicate; values in brackets are the standard deviation of the measurements. C and N analysis was not duplicated.

Table 2.3 Correlation (r) between characteristics of prunings.

	%N	%PP	%L
%N	1.00		
%PP	0.37 ns	1.000	
%L	0.35 ns	0.99 *	1.000

* - Significant at $P \leq 0.05$
n.s. - Not significant at $P \leq 0.05$

Table 2.4 Chemical characteristics of *Inga* and *Centrosema* when grown in pure and mixed stands.

	% N*	% PP*	% L*
Inga pure	3.58	3.78 (0.001)	34.8 (2.6)
Inga mix	3.63	3.86 (0.001)	34.0 (0.1)
Centrosema pure	3.29	1.46 (0.001)	15.6 (0.8)
Centrosema mix	3.13	1.28 (0.041)	18.1 (0.8)

* Lignin (L) and polyphenol (PP) analysis completed in duplicate; values in brackets are the standard deviation of the measurements. N analysis was not duplicated.

Table 2.5 Chemical characteristics of the Mix employed in comparison with:
a) 39/61 proportions rather than perfect 50/50 and,
b) change in chemical characteristics from pure to mixed stands.

	%N	%PP	%L	L+PP:N	C:N
Measured values for the Mix used (39/61 *Inga* / *Centrosema* from mixed stands)	3.32	2.28	24.3	8.0	13.9
Calculated expected values for mixed material based on 39/61 *Inga* / *Centrosema* from pure stands	3.40	2.36	23.1	7.5	13.6
Calculated based on 50/50 *Inga* / *Centrosema* from mixed stands)	3.38	2.57	26.1	8.5	13.8

Proportion of NH_4^+ in PL samples compared to SL samples:

Samples were analyzed for NH_4^+ and NO_3^-. The term "mineral N" is used to designate combinations of these two N ions. Nitrite was assumed to be present only at very low concentrations. Mineral N was collected by plate lysimeters predominantly as NH_4^+ and mineral N sampled in soil solution by SLs was almost totally NO_3^- (Figure 2.2). Prunings on PLs released between 60 and 98 % of their mineral N as ammonium; this percentage varied with time although does not form a trend. With the exception of *Colubrina*, all pruning treatments on PLs released similar proportions of their mineral N as NH_4^+ (87%, standard deviation 10%). *Colubrina* released 64% (standard deviation 34%) of its mineral N as NH_4^+ (data not shown).

In soil solution mineral N sampled was between 2.8 and 13.5 % NH_4^+. The proportion of mineral N as NH_4^+ declined steadily with time: on days 12 and 19 prunings released a significantly larger proportions than the 3 later sampling dates (Figure 2.2).

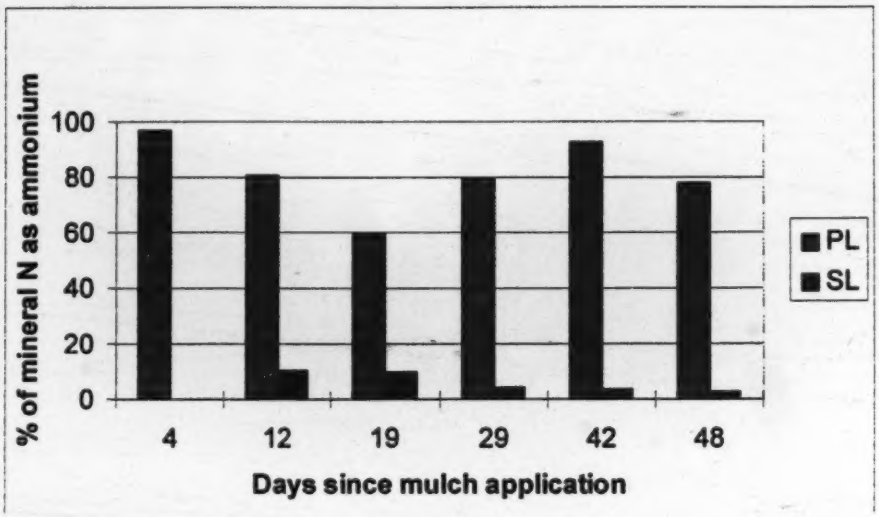

Figure 2.2 Percent of mineral N as ammonium from plate lysimeters (PL) and suction lysimeters (SL). Mineral N considered to be NO_3^- plus NH_4^+.

Mass loss from prunings in plate lysimeters (PLs):

Mass loss from prunings in PLs was established for the 54 total days of decomposition. Mass loss occurred in the following order: *Centrosema* (61%), Mix (56%), *Inga* (38%) and *Colubrina*(34%) (figure 2.3). A prediction was made for mass loss of the Mix treatment based on the mass loss measured in *Inga* and *Centrosema* individually. The predicted mass loss for the mix is slightly lower than, but not significantly different from, that observed. For all treatments, mass loss was significantly correlated to L+PP:N ratio (r^2 = .56, 99% confidence level) but not to C:N ratio (r^2 = .10).

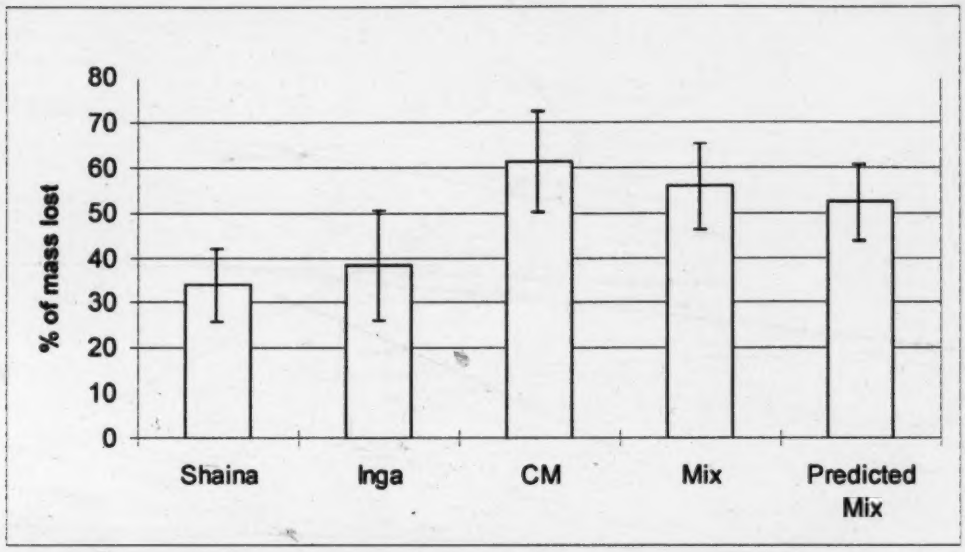

Figure 2.3 Mass loss of prunings during 8 weeks decomposition. Shaina = *Colubrina glandulosa*, *Inga* = *Inga edulis*, CM = *Centrosema macrocarpum*, Mix = *Inga* & *Centrosema*, Predicted Mix = N release from Mix calculated by the weighted mean of mass lost from mix components in pure form (prediction is based on assumption of non-interaction between components).

N release from prunings in plate lysimeters (PLs):

Mineral N release from the prunings in PLs was taken to represent N mineralization in the mulch layer, measured at the mulch/soil interface. To correct for differences in the amount of dry matter applied, and for differences in the % N content of the prunings, N release results were calculated as percentages of the original amount of N applied. A predicted value was produced for the Mix treatment, based on N release from the Mix components, *Inga* and *Centrosema*, in pure form. To correct for the amount of N present in rainwater and through-fall, and for N in soil splashed into the plate, control plates (with no prunings) were used (N concentrations in PL controls were always very low - data not shown). Values shown are the difference between treatments and control plates (Figure 2.4).

In general, N release declined with time with the exception of day 47, the last measurement date. Relative to the other treatments, *Colubrina* released very small amounts of N; on days 18 and 47 negative values are shown for *Colubrina* meaning that more N was found from control plates than *Colubrina* plates. *Centrosema* and *Inga* exhibit very similar patterns of declining N release over time. *Centrosema* released more mineral N than *Inga* at all times although this difference was not always significant. In the Mix treatment, decline in N release was not as rapid or consistent as in the other treatments; on days 28 and 47 prunings released larger amounts than their preceding measurement date. Compared to a predicted release pattern for the Mix, the observed release was lower on day 18 and higher on day 28.

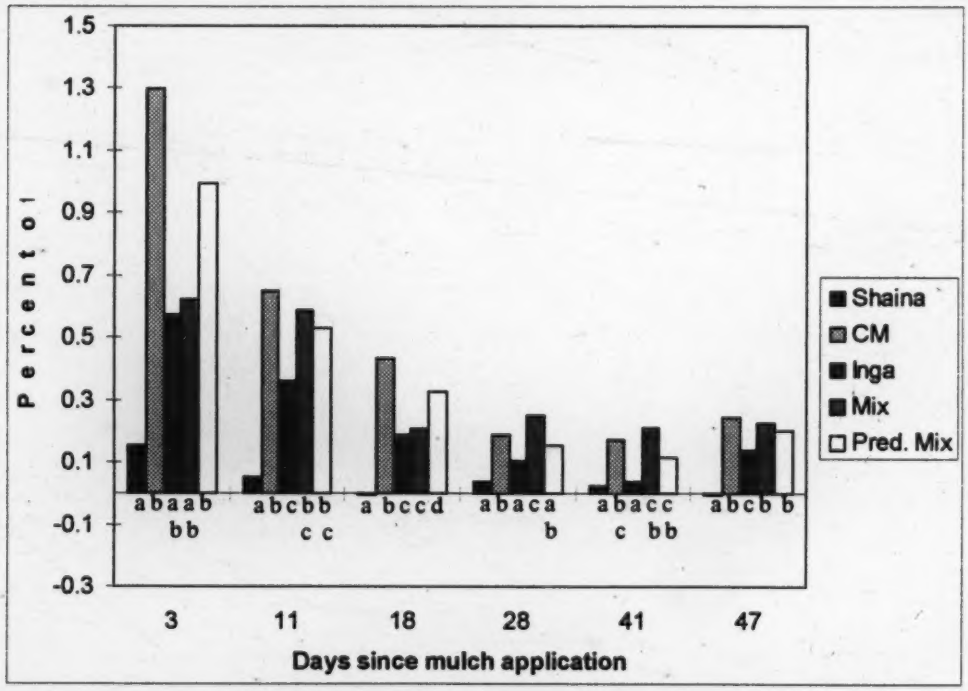

Figure 2.4 Mineral N collected from prunings in plate lysimeters at discrete dates during decompositon. Shaina= *Colubrina glandulosa*, CM= *Centrosema macrocarpum*, *Inga*= *Inga edulis*, Mix= 39/61 proportions of *Inga*&CM respectively, Pred. Mix= Calculated N release from Mix under the assumption of non-interaction between components. LSD procedure was used to separate means; columns labeled with the same letter on a given day are not significantly different (P≤0.05).

Regressions between % N release and the initial chemical characteristics of prunings were conducted. Only L+PP:N ratio and C:N ratio are compared here because lignin and polyphenols were highly correlated in the materials used. L+PP:N ratio was a good predictor of N release to day 18 (Table 2.6). L+PP:N was a better predictor of N release than C:N ratio at all times. On days 28 and 41 none of the initial chemical characteristics had correlation coefficients greater than .60 and thus explained no more than 36% of the variation in N release. At the last measurement date (day 47) there was an overall improvement in correlation of N release with initial chemical characteristics. To further elucidate the behavior of the Mix treatment, correlation coefficients were calculated with the Mix data excluded, and compared to the coefficients calculated from the full data set. With Mix data absent L+PP:N is a better predictor at both the beginning and end of the 7 week period (Table 2.7).

Table 2.6 Correlation coefficients (r) between incremental % of pruning N released (PLs) and chemical characteristics of prunings.

Sample day	3	11	18	28	41	47
L+PP:N †	-.90***	-.80***	-.83***	-.60***	-.54**	-.77***
C:N †	-.58**	-.58**	-.54***	-.40 ns	-.23 ns	-.65***

*** significant (P≤0.01)
** significant (P≤0.05)
ns not signigicant (P≤0.05)
† L+PP:N = lignin + polyphenol to nitrogen ratio. C:N = carbon to nitrogen ratio.

Table 2.7 Correlation coefficients (r) without Mix. Correlation between incremental % of pruning N released (PLs) and chemical characteristics of prunings.

Sample day	3	11	18	28	41	47
L+PP:N †	-.95***	-.80***	-.86***	-.70***	-.58**	-.85***
C:N †	-.62 ns	-.61**	-.60***	-.46 ns	-.22 ns	-.74***

*** significant (P≤0.01)
** significant (P≤0.05)
ns not signigicant (P≤0.05)
† L+PP:N = lignin + polyphenol to nitrogen ratio. C:N = carbon to nitrogen ratio.

Plate lysimeter (PL) mesh size:

Mesh size imparted a significant difference in N release from the Mix but not from any of the other treatments (Table 2.8). In the Mix, the large mesh released more N on average (0.11% of added N) than the small mesh. Mass loss did not differ significantly with mesh size in any treatment.

Table 2.8 Tests for significance of difference in mineral N release due to mesh size.

	Difference † (mean % N release)	Probability > | T | ††
Colubrina	-0.03	0.1393
Inga	0.02	0.5217
Centrosema	0.03	0.2730
Mix	0.11	0.0071

† Values represent the mean difference in % N release from meaningfully paired instruments (large size mesh versus small sized mesh).

†† A probability > 0.05 indicates that there is no difference attributable to mesh size.

Mineral N sampled by suction lysimeters:

Values presented for mineral N in soil solution, as sampled by suction lysimeters, are the difference between treatment and control plots (Figure 2.5). The difference between treatment and control plots was considered to be N mineralization from the pruning treatment. Although lysimeter data were found to be highly variable, they suggest treatment specific patterns of mineral N in soil solution. Under *Colubrina* and *Inga* prunings, mineral N in soil solution declined steadily with time and *Colubrina* results are actually negative (less N under treatment than under control) after day 20. Under *Centrosema* prunings, mineral N is constant or slightly increasing to day 30 and then declines. Compared to a calculated predicted value for the Mix, the observed values are

significantly lower up to day 30 and are higher (although not statistically significant) on days 44 and 50. Note that in the analysis of covariance method used to separate treatments, the Mix was unique with a significantly positive slope (increasing N release over the measurement period). All other treatments had either 0 or negative slope (data not shown). An example of the analysis of covariance procedure is presented in Appendix 1. Time was considered to be the covariant.

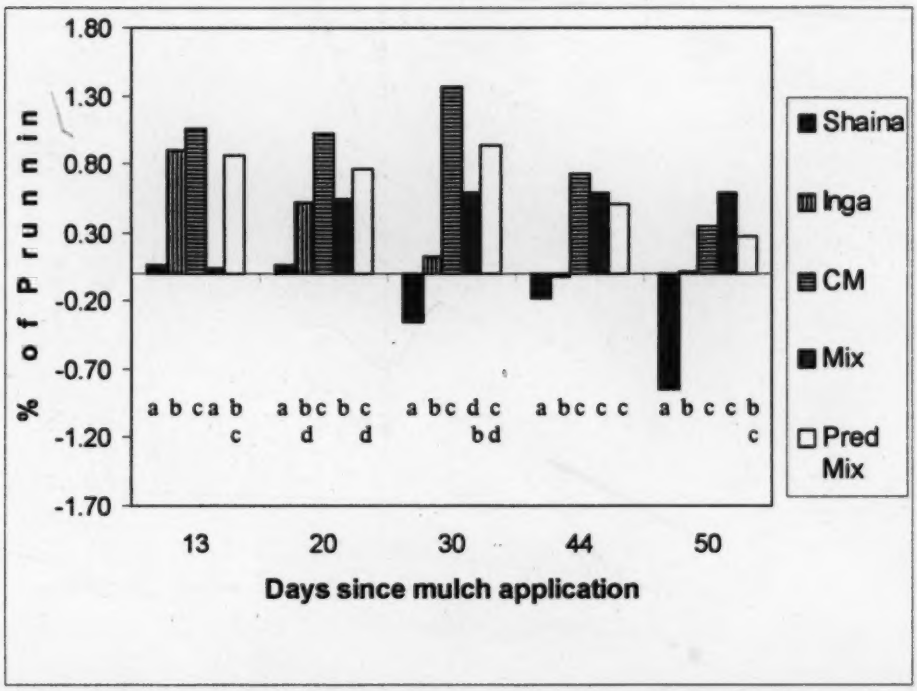

Figure 2.5 Percent of added pruning-N measured in SL samples at 5 discrete dates. Shaina=*Colubrinaglandulosa*, *Inga*=*Inga edulis*, CM=*Centrosema macrocarpum*, Mix=39/61 proportions of *Inga*&CM respectively, Pred Mix=calculated release from Mix under the assumption of non-interaction between mix components. Means on a given day separated by analysis of covariance (Appendix 1). Columns labeled with the same letter are not significantly different (P≤0.05).

The correlation between % of pruning N measured in soil solution and initial chemical characteristics of the prunings is presented in Table 2.9. L+PP:N ratio was the best

predictor of N release, well correlated with N in soil solution at days 20, 30 and 44. None of the chemical parameters was well correlated with N in soil solution at the first measurement point. At the last measurement, day 50 after pruning application, the correlation between C:N ratio and N in soil solution improved to become significant and was about equal to L+PP:N as a predictor of N release. Correlation coefficients improved at the beginning and end of the decomposition period when Mix data are not included (Table 2.10).

Table 2.9 Correlation coefficients (r) between % of pruning N measured in SL samples and chemical characteristics of prunings.

Sample day	13	20	30	44	50
L+PP:N †	-.34 ns	-.73 ***	-.88 ***	-.76 ***	-.67 **
C:N †	-.44 ns	-.53 ns	-.48 ns	-.38 ns	-.70 **

*** significant at 99% confidence level
** significant at 95% confidence level
ns not significant at 95% confidence level
† L+PP:N = lignin + polyphenol to nitrogen ratio. C:N = carbon to nitrogen ratio.

Table 2.10 Correlation coefficients (r) without Mix. Correlation between % of pruning N measured in SL sample and chemical characteristics of prunings.

Sample day	13	20	30	44	50
L+PP:N †	-.54 ns	-.78**	-.88***	-.79**	-.70**
C:N †	-.66 ns	-.57 ns	-.47 ns	-.34 ns	-.74**

*** significant at 99% confidence level
** significant at 95% confidence level
ns not significant at 95% confidence level
† L+PP:N = lignin + polyphenol to nitrogen ratio. C:N = carbon to nitrogen ratio.

2.4 Discussion

Predominant form of mineral N released from prunings in contrast to samples of soil solution:

Differences in the predominant form of mineral N in the mulch (ammonium) versus soil solution (nitrate) indicate that ammonium was rapidly nitrified upon entering the soil matrix. In the mulch, high proportions of mineral N present as ammonium suggest that ammonification was much more rapid than nitrification. Apparently the leaf and petiole surfaces of the mulch layer do not support large populations of nitrifiers relative to other decomposers. It is possible that fungi account for much ammonification in the mulch where bacterial survival (hence most nitrifiers) is limited. Fungi differ from bacteria in their ability to bridge extreme environments with hyphae. The differing rates of nitrification in the mulch versus the soil matrix indicate different microbial enviroments: the mulch layer probably experiences moisture and temperature extremes on a diurnal basis while conditions in the soil are strongly buffered. In soil solution, the concentration of NH_4^+ declined with time as the rate of its addition to the soil slowed.

Control of N release by chemical characteristics of the prunings:

It was hypothesized that PP:N or L+PP:N ratio would be the best predictor of N release in these materials. The near perfect correlation between lignin and polyphenol contents precludes strong distinction between phenolic versus lignin induced reductions in mineralization rates in the group of materials used. L:N and PP:N were very similar to L+PP:N as a predictors of N release (See Appendix 7). L+PP:N was a better predictor than C:N. Other investigations have found L+PP:N to be the best predictor of N release (Fox et al. 1990, Constantinides and Fownes 1994, Handayanto et al. 1994, 1995 and 1997, Lehmann et al. 1995).

N release rates from tropical agroforestry litters and prunings was investigated widely in the last decade (Fox et al. 1990, Palm and Sanchez 1990 and 1991, Oglesby and Fownes

1992, Tian et al. 1992 and 1993, Xu et al. 1993, Constantinides and Fownes 1994, Handayanto et al. 1994, 1995 and 1997, Lehmann et al. 1995, and others). One of the common objectives is to find a robust predictor of N release that is valid across diverse types of plant materials and sites, but work has been dominated by laboratory incubations. A working predictor of N release must also be tested in the field where temperature and humidity vary, and diverse biotic communities (including meso and maco fauna) are present. The finding in this field experiment that L+PP:N is significantly correlated with N release from the mulch layer and with N in soil solution suggests that L+PP:N ratio may in fact be highly applicable to working agroforestry systems and indeed a robust predictor of N release.

Various mechanisms are suggested for reduced mineralization or stabilization of N compounds by phenolics, including binding reactions with organic N compounds, toxicity to microbes and fauna, and nitrosation. During nitrification nitrite may combine with phenolics forming stable complexes in a reaction called nitrosation (Nelson and Bremner 1969; Azhar El Sayed et al.. 1986). During oxidative coupling or condensation reactions in the soil, phenolics and/or quinones also form strong covalent bonds with amino groups (Sjoblad and Bollag 1981). In another type of binding reaction between polyphenols and proteins, hydrogen bonds (weaker than covalent bonds) form between H atoms bonded to strongly electonegative moeities (OH and NH groups) and other nucleo-philic groups (Appel 1993; Reed 1995). Binding reactions with organic N groups may inhibit decomposition not only by rendering N rich substrate unavailable, but also by interference with the action of extracellular enzymes (Benoit et al. 1968; Benoit and Starkey 1968; Leak and Read 1989). Interestingly, Benoit et al. (1968) conclude that enzyme inactivation was more important than binding of N compounds in tannin inhibition of decomposition. The reactivity of phenolics can also be toxic to decomposer organisms. Astringency or binding of proteins causing enzyme inhibition and substrate deprivation leads to toxicity (Scalbert 1991; Reed 1995). Phenolics have also been linked to inhibition

of oxidative phosphorylation in mitochondria and destruction of cell membranes (Scalbert 1991).

Phenolics originating from plant materials are soluble to varying degrees during decomposition (Shindo and Kuwatsuka 1975; Handeyanto et al. 1994). It is possible that reactive phenolics leach from one material to influence N release in another, when different types of prunings or litters are mixed. The potential for early inhibition of decomposition by polyphenols is therefore distinct from lignin which is likely insoluble until late stages of decomposition.

Lignin is a structural component of plant cell walls and is resistant to decomposition; it is equated with "low quality" in plant materials by the TSBF programme (Anderson and Ingram 1989) and considered an important physicochemical control on decomposition (Meentemeyer 1978). Phenylpropane (phenolic) units combine with each other in a variety of cross-linkages leading to a very large and complex lignin polymer. The complexity and lack of repeating pattern in the polymer, combined with the reactive yet stable nature of the benzene ring, make lignin resistant to decomposition. White-rot fungi are the only organism known to be able to degrade lignin completely, from de-polymerization to ring cleavage. Bacteria are not believed able to cleave the benzene ring in molecules composed of more than two benzene rings (Odier and Artaud 1992).

As lignin depolymerization occurs smaller units can be oxidatively activated and recombine - possibly stabilizing N compounds between recombining units. More labile carbohydrates and proteins may be complexed by lignin in the plant cell and physically protected from enzymes. Physical protection may also be expressed at a cellular level, where labile materials inside the cell are not available to decomposers before lignin in the cell wall is breached. Fyles and McGill (1986) found that cutting pine needles into centimetre long pieces doubled their decay rate. Control of decomposition by lignin may not be predictable by the quantity of lignin in the material - the physical structure of the

material, and location of the lignin, may be more important. There are a number of examples of plant materials behaving in the opposite manner from what their lignin:N ratios predict. Palm and Sanchez (1991) found slow mineralization in *Leucaena leucocephala* despite a low L:N ratio and conversely Oglesby and Fownes (1992) noted that *Sesbania sesban*, with high lignin content, mineralized rapidly.

In this experiment *Inga edulis* released mineral N rapidly in the first 2 weeks of decomposition in spite of a high L:N ratio (almost 10), similar to that of *Colubrina*. Further, correlation coefficients for L+PP:N ratio and N release in the first 3 weeks improve when *Inga* data are not included (data not shown). N release from *Inga edulis* may be a two stage pattern, with some immediate release and another stage of release much later (D.C.L Kass, personal communication). In some white rot fungi degradation of lignin is believed to be repressed by available N (Reid 1990; Van der Woude et al. 1993). In this experiment N release from *Inga* prunings in PLs declined to day 41 and increased on day 47 - could N repression of lignin metabolism have contributed to this pattern?

Analysis of plant materials for lignin content is not specific to the phenylpropane units which compose lignin, and Preston et al. (1997) argue that this is a serious oversight in many decomposition studies to date. Lignin analysis may be inaccurate as acid-insoluble constituents which are not lignin, cellulose or ash may be included in the acid detergent fibre method most commonly used by agroforestry workers (Anderson and Ingram 1989).

Polyphenols comprise a wide array of chemical structures, some participating in binding reactions to a much greater extent than others. The Folin-Denis method which is most commonly used to analyze for polyphenols in litter quality analysis does not differentiate for protein binding. Protein binding capacity (PBC) is used to characterize pruning and litter phenolics as an alternative to the Folin - Denis method (Handayanto et al. 1994 and 1997). The assay employs bovine serum albumen (BSA) on chromatographic paper, to

which extracted phenolics are applied and the quantity of BSA bound is quantified. Handayanto et al. (1994 and 1997) have found that PBC is not always consistent with polyphenol content by the Folin-Denis method, and that PBC may be the best predictor of N release in some situations. Interestingly, they found that PBC was the pruning characteristic most closely related to maize N recovery from a range of mixes (high, medium, and low PBC).

If protein binding mechanisms were the cause of the apparent interaction in the Mix treatment and the recalcitrant nature of *Inga* prunings, then PBC may have been a useful measure in this experiment. PBC assays were not conducted in this experiment, and it is not clear to what extent they were correlated to polyphenol contents measured by the Folin-Denis method. PBC may have allowed for differentiation between the action of soluble polyphenols and the recalcitrant nature of lignin.

Tests for inter-species interaction in the pruning mixture:

It was hypothesized that when *Inga* and *Centrosema* prunings were mixed, N release from *Centrosema* would be slowed due to the action of soluble phenolics from *Inga*; that is, there would be interaction between the pruning species. The null hypothesis, that there is no interaction, would mean that N release from a mix could be predicted by an arithmetic mean weighted for the quantity of each mix component and based on each component's individual release rate.

The overall pattern of N release from both PLs and SLs was initially less than, and later greater than, the null hypothesis would predict, suggesting that interaction occurred. Initial reductions in N release from the Mix are consistent with the action of soluble polyphenols. Although the sustained, higher than predictable levels of N release from the mix in the last 2 (SLs) to 3 (PLs) weeks of decomposition are the opposite of what could be expected by phenolic action, they could be explained by temporary stabilization in protein-phenol complexes: mineralization is delayed for a period of 2-4 weeks.

In the limited documentation of nutrient release from decomposing mixes there is evidence for both interaction and lack of interaction (Myers et al. 1994), and little agreement on the conditions under which interaction may occur. Palm (1995) argues that there isn't definite evidence of altered patterns of nutrient release when "high" or "low" quality materials are mixed, but that a pattern paralleling the high quality component is observed. Handayanto et al. (1994) suggested that phenolic binding reactions may be less important under leaching conditions, where phenolics would be washed from prunings or litters. In contrast Handayanto et al. (1997) found that PBC was more important under leaching conditions, and they suggest that phenolic protein binding depends on solubility of interacting constituents. Solubility is also a control on substrate availability to microbes, and is likely to be critical to many decomposition phenomena. The opportunities for binding reactions may be very different in a mulch layer versus a soil matrix, because dissolved organic constituents are probably more mobile in a mulch layer than in the soil.

Montagnini et al. (1993) studied the decomposition of a mixture of contrasting materials in a litter bag experiment using high N *Stryphnodendron microstachyum* and low N *Vochysia ferruginea* (lignin, polyphenol or protein binding were not measured). They found that the high N component decomposed significantly faster in a mixture than in pure form during 15 - 75 days decomposition, but note that there were no differences after 90 days. The low N component in their study also lost more mass in mixture than in pure form, and although they note that the difference was never significant, the trend toward a difference continued to increase to the end of the 3 ½ month experiment and may have become significant later on.

In this experiment the Mix was the only pruning treatment which released N more rapidly from PLs under a large sized mesh compared to a small sized mesh. Although the 0.11% difference seems small, note that because time is not considered in the mesh size analysis, each difference has 18 potential replications (3 replicates X 6 samplings). Also, prunings

on PLs nearly always released less than 1 %, and often less than 0.5 %, of their N per rainfall event, thus a 0.11% difference is plausible. The large mesh (3.5 mm) presumably allowed meso-faunal access while the small mesh (0.5 mm) allowed only micro-faunal access. None of the agroforestry literature on decomposition of mixes discuss faunal attack. In this experiment the mixed environment may have supported greater meso-faunal activity leading to faster N release. Faunal activity is connected with increased mineralization rates in both temperate (Williams and Griffiths 1989; Huhta et al. 1989) and tropical (Tian et al. 1992 and 1995) environments. Williams and Griffiths (1989) also related faunal activity to increased leaching of ammonium and nitrate.

Janzen et al. (1995) suggest that community level controls on decomposition exist at a higher hierarchical level than physicochemical or population level controls. Citing Blair (1990), Smith et al. (1998) suggest that mixed litters support greater microbial diversity and faster decomposition. Aside from mineralization of temporarily stabilized phenolic complexes, the sustained, unpredictably high rates of N release after 3-4 weeks of decay are difficult to explain on the basis of initial chemical characteristics of the materials. A community level control on decomposition could be responsible. The mixed environment may have supported greater microbial or faunal activity in later weeks of decomposition, as suggested by results from altering the mesh size over the plates.

2.5 Conclusions

L+PP:N ratio was significantly correlated with N release from prunings placed within an improved fallow agroforestry system. On average, however, L+PP:N only explained about 55% of the variation in N release to leachate as measured by plate lysimeters or porous cup suction lysimeters; prediction of N release in agroforestry management decisions will require more precision.

Interaction occurred and seemed to result in a mineralization delay of 2-4 weeks. Interaction may have occurred through a physicochemical control such as phenolic binding reactions, a population level control such as decomposer toxicity, a community level control, or a combination of these. Community level controls, including faunal attack, are complex and poorly documented but may be significant phenomena in ecosystems.

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3. Soil mineral N distribution following slash and burn disturbance of improved fallow vegetation in the Humid Tropics of Peru

3.1 Introduction

It is suggested that fire was the first great tool which humans used to alter their environment (Stewart 1956). Fire remains an integral component of shifting cultivation, or slash and burn agriculture, which is estimated to be currently practiced on 30% of the worlds arable soil and sustains approximately 300 million people (Bandy et al. 1993). Whether in shifting cultivation or other production systems, fire is powerful, relatively easily employed, and its use widespread. Forest loss through shifting cultivation in the Humid Tropics is suggested to be a significant contribution to global warming (EPA 1990) and it is criticized as a cause of soil degradation.

Fire perturbs nutrient cycles by killing site vegetation and rapid conversion of plant biomass from organic to mineral forms. Vegetation death stops the plant mediated element transfers which normally occur between soils, plants, and the atmosphere. In wildfires, burning converts plant organic matter into mineral constituents and charred organic particles. A portion of the minerals released during burning are volatile and transferred to the air, others compose residue ash which is deposited on the soil surface. It is suggested that fire can improve soil fertility by mobilizing nutrients and speeding mineralization rates. Rapid plant uptake of mobilized nutrients and invasion by N fixing species are common after fires. These responses to fire disturbance mitigate nutrient loss from the ecosystem (Woodmansee and Wallach 1981; Singh 1994). Among the factors which control the extent to which nutrient cycles are disturbed as a result of burning is the distribution of organic matter within the soil-plant system. In systems such as grasslands, where a high proportion of system organic matter is in the soil, volatile and particulate

nutrient losses during burning may be small relative to total nutrient stocks of the soil-plant system.

During combustion, N is generally believed to be volatilized and lost from soil-plant systems. There are many references in the literature, however, to the presence of N in ash. Christensen (1973) concluded that increased soil nitrate concentrations in a burned area of Chaparral ecosystem were due to the addition of ammonium and organic N in ash. Weston and Attiwill (1996) found a 2.5 fold increase in soil mineral N concentrations after burning of mature *Eucalyptus regnans* forest in Australia, and suggest that mineral N increases are attributable to mineralization by burning rather than microbial action. Working at Yurimaguas, Seubert et al. (1977) found 1.72% (mass basis) total N in ash (including fine ash and larger charred particles) after burning a 17 year old stand of secondary forest. Sanchez (1987) cites work completed at Yurimaguas, Peru and Manaus, Brazil where ash following burning of various forest and fallow types contained 9 - 17 g total N per kg of ash. Bandy et al. (1993) indicate that about ½ of the original N is retained in ash when vegetation is burned in slash and burn systems. Note that Seubert et al. (1977), Sanchez (1987), and Bandy et al. (1993) do not differentiate between mineral and organic N in their analysis of ash, and included charred particulate matter in their analysis. The presence of NH_4^+ in ash is also suggested by Woodmansee (1981) and Klopatek et al. (1990).

There appears to be increasing consensus among some investigators that volatile N loss during burning is directly proportional to mass loss of organic matter (O'Connel and McCaw 1997; Gillion et al. 1995; Raison et al. 1984), suggesting that little mineral N can be contained in ash. Burning of organic matter involves initial heating and evaporation of water, pyrolisis (or gasification due to heating), and oxidation of pyrolysate gases. During pyrolysis heating causes homolytic cleavage of C-N bonds releasing volatile amine radicals (NH_2) (Tillman et al. 1981). Tillman et al. (1981) suggest that O_2 supply may be a major control on the ultimate N forms evolved during burning of organic matter: if amine

radicals are immediately exposed to sufficient O_2 (such as in some engineered suspension firing systems) higher yields of NO, rather than N_2, occur. In burning of a fuel bed (such as occurs in wildfire), an O_2 poor pre-combustion zone allows reduction of amine radicals to N_2. Even when amine radicals are passed into the primary combustion zone, they may be ultimately reduced to N_2 through a variety of intermediate dependent pathways (figure 3.28) (Tillman et al. 1981; Blint and Dasch 1984). DeBell and Ralston (1970) investigated forms of volatile N loss from forest litters during burning and concluded that in most cases 99% of N release due to heating is in the form of N_2. Raison (1979) summarizes that ash typically contains only small amounts of mineral N but notes that some studies of ash mineral composition are completed in ovens where burning is more complete than may occur in the field. Non-explicit definitions of ash are a major constraint to comparing reports of N fate during burning.

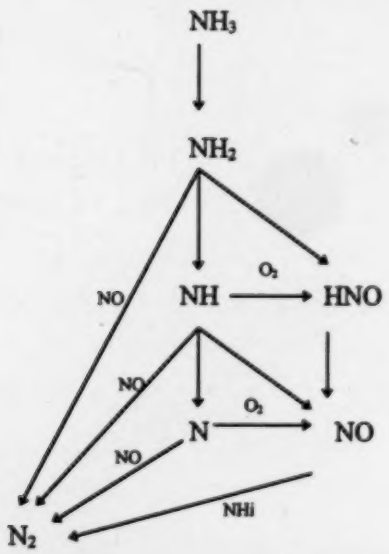

Figure 3.6. Pathways of ammonia combustion. NHi=amine radicle. Source: Blint and Dasch 1984.

Fire modification of nutrient flows in the Humid Tropics is important, but may not be well characterized for a number of reasons. 1) Investigation of fire with emphasis on nutrient cycles is dominated by work in dry or temperate region ecosystems. 2) The ecosystem changes imparted by fire are notoriously variable depending on characteristics of vegetation, soil, fuel, climate and weather, topography, and fire intensity and frequency (Richter and Ralston 1982). 3) Leaching losses following burning in the Humid Tropics are potentially greater than in the Arid and Semi-arid tropics, and temperate regions, which may receive less than a tenth of the precipitation. 4) In humid tropical forests organic matter may be distributed to a greater degree above the mineral soil surface, where its loss could significantly reduce system reserves.

The objectives of the work reported here were:

- To characterize mineral N distribution within the soil profile following slash and burn disturbance at a humid tropical site in the upper Amazon basin of Peru.
- To test the hypothesis that slash and burn disturbance would result in N leaching loss.
- To compare the utility of ion exchange membranes and suction lysimeters to assess soil N status, and then to relate N status to soil fertility and maize yields.

3.2 Materials and Methods:

Site Characteristics:

The experiment was conducted on the same improved fallow site described in Chapter 2; a Typic Paleudult on an area of upland terrace (Tyler 1975) which had been abandoned by a local farmer. Soil bulk densities are presented in Table 3.1, and soil moisture retention curves are presented in Figure 3.1.

In October 1994 ICRAF acquired the land and established the improved fallow vegetation as described in Chapter 2. The fallow phase of the system was planned to last 3 years before initiation of annual crops. Soil physical and chemical characteristics were

monitored by ICRAF in 1994-1996 and data for leaching of NH_4^+ and NO_3^- were recorded (Figures 3.2 and 3.3). Nitrate leaching to 50 and 100 cm depths was greater during the first rainy season after fallow establishment compared to the second. NH_4^+ leaching was an order of magnitude less than that of NO_3^- and was sporadic.

Table 3.1 Soil bulk density by layer.

Soil layer:	Bulk Density (Db):
0-10 cm	1.06
10-20 cm	1.34
20-30 cm	1.29
30-50 cm	1.36
50-100 cm	1.40
100-150 cm	1.53

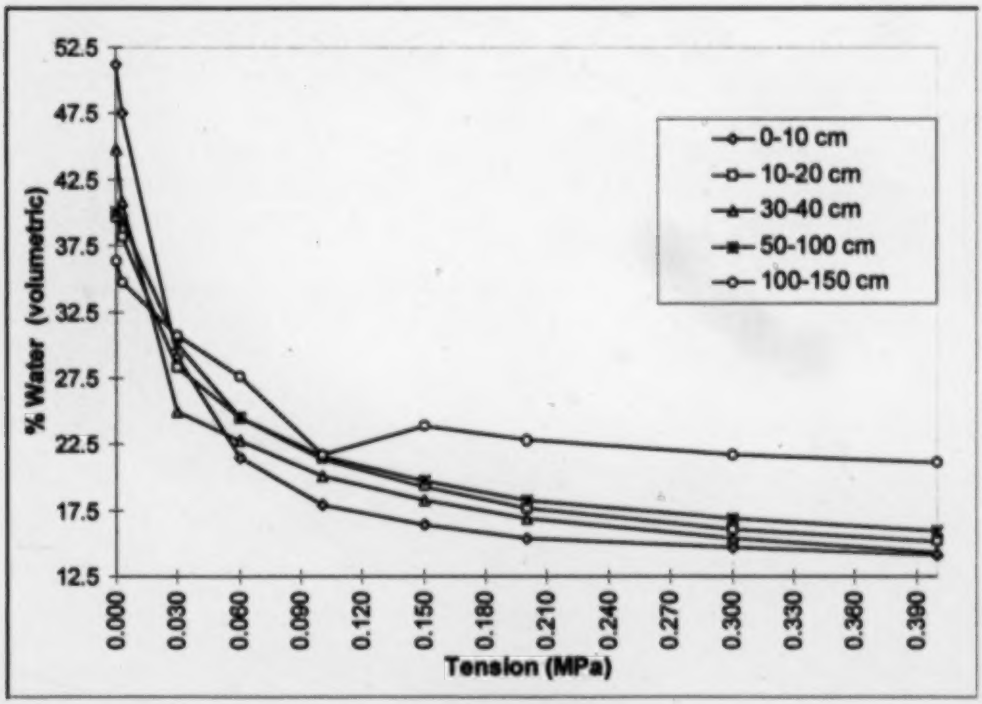

Figure 3.1 Water retention curves for improved fallow site at Yurimaguas, Peru.

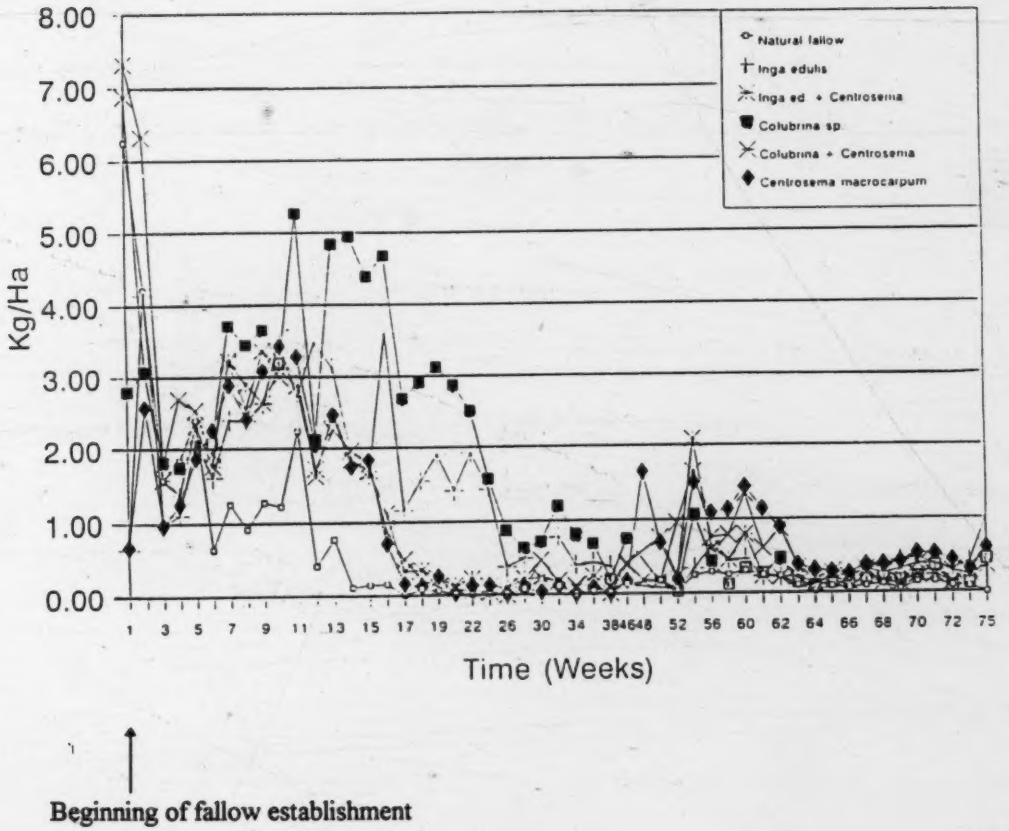

Figure 3.2 Nitrate measured at 50 cm depth under various fallow treatments. Source: JC Alegre, ICRAF, personal communication.

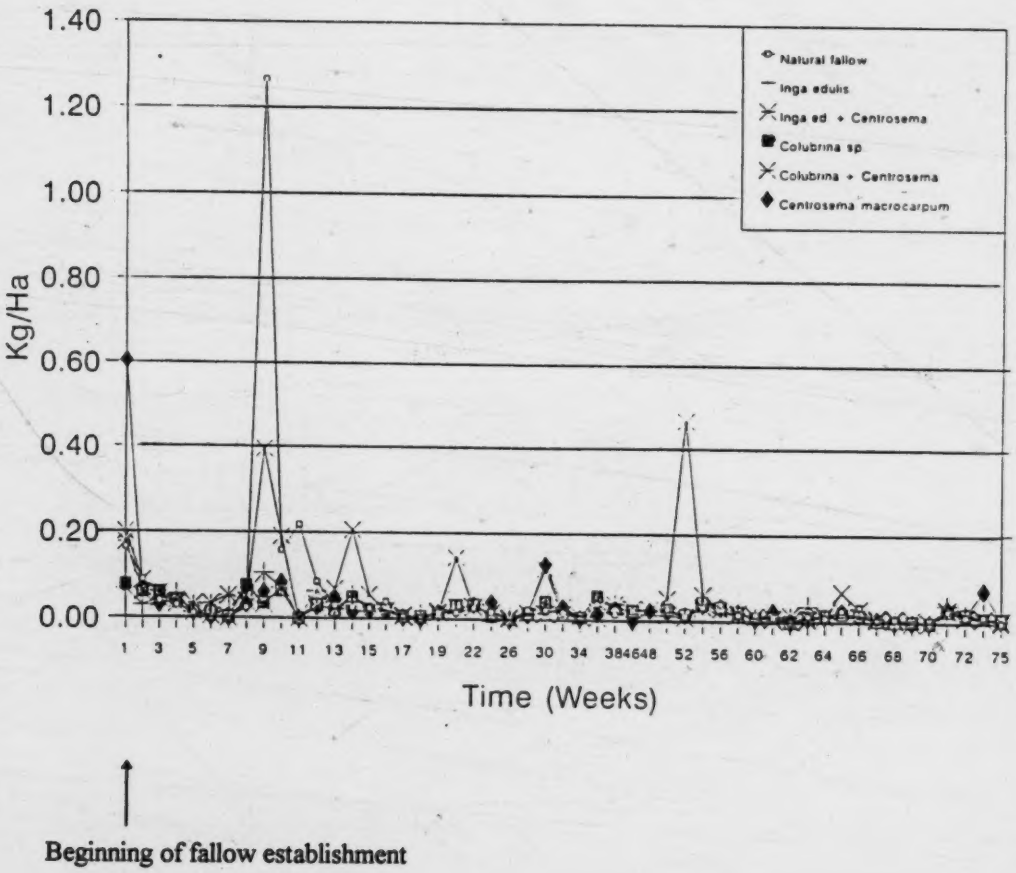

Figure 3.3 Ammonium measured at 50 cm depth under various fallow treatments. Source: JC Alegre, ICRAF, personal communication.

Slash and Burn Disturbance and Maize Planting:

The 3 year fallow stage was completed in 1992, after which trees on site were felled by chain-saw and smaller vegetation felled by machete. *Colubrina* logs were harvested as construction poles and all other wood pieces greater than 5 cm diameter were harvested for firewood. All remaining fine slash and foliage was left on site for broadcast burning. Litter was dried for one week and was manually turned to promote homogenous drying. The site was broadcast burned on 13 November (See Appendix 5, photos 5 & 6). Although pre-burn masses of organic matter were not measured, *Colubrina* plots had visibly less pre-burn litter than the others. Surface organic matter reductions due to burning were approximately 90% (See Appendix 5, photos 7 & 8). *Colubrina* plots did not have sufficient fuel continuity for contiguous fire spread and many patches were not burned. In *Centrosema* plots a layer of intact, non-burned litter remained below the ash of the burned material. 18 hours following burning, the site was planted with a local maize variety (See Appendix 5, photos 9 & 10). A planting stick was used to open a hole 3-5 cm deep, 3 maize seeds dropped were in, and the hole was lightly covered.

Suction Lysimeter Installation and Sampling:

Porous (ceramic) cup suction lysimeters were installed 4 days following burning. Ceramic cups were prepared by washing, soaking in dilute HCl, and soaking in distilled water as described in Chapter 2. Lysimeters were located near the centre of each experimental plot at depths of 15, 30, 50 and 100 cm; instruments were spaced at least 2 meters from each other forming a square. Prior to installation, each ceramic cup was dipped in a thick sand/deionized water slurry; the same slurry was poured around the lysimeter tube after insertion. The sand used for slurring was collected from a nearby roadside cut-bank. Following the addition of slurry, lysimeters were gently packed into place with dry earth. Samples of the sand used for slurry were taken for mineral N analysis.

During or shortly after moderate or heavy rains, lysimeters were evacuated to 0.05 Mpa (50 centibars) tension. No more than one sampling was conducted per work week, but

the interval between samplings depended on rainfall frequency. Mean sample interval was 6.4 days, with a minimum of 4 days and a maximum of 11 days. 24 hours following vacuum application, water samples were removed from lysimeter tubes and immediately transported to the laboratory. The quantity of water extracted from lysimeters varied over time and between instruments. Volumes extracted at each sampling were recorded but are not a component of these results because only mineral N concentrations are reported. Samples were stored under refrigeration and were normally analyzed the day following extraction; samples were never stored longer than 3 days.

Ion Exchange Membrane Sampling:

Resins fabricated in membrane form with the capacity for either anion or cation exchange (ion exchange membranes or IEMs) were employed. IEMs marketed as Plant Root Simulators ® were leased from Western Ag Innovations of Saskatoon, Saskatchewan. Plant Root Simulators have 1.7 X 4.0 cm of membrane held by a rigid plastic frame at the membrane periphery; the plastic frame and membrane form a flat, pointed probe which can be inserted into and extracted from the soil with minimal soil disturbance. Ion exchange membranes were installed in soils of *Centrosema*, *Inga*, *Colubrina*, and Natural fallow treatments. Membranes were not installed in soils of the 2 mixed species treatments due to lack of instruments. A shallow access hole was excavated and IEMs were inserted into the face of the hole with their long axis horizontal at 2 and 15 cm depths. The hole was covered to prevent it filling with water or eroded soil. The ion exchange membranes were conditioned in the lab by soaking for 1 hour in 0.5 M $NaHCO_3$. Following 7 days in the field, membranes were removed from the field, soaked individually for one hour in 20 ml 0.5 M HCl, and the extractant analyzed for mineral N.

Laboratory Analysis, Data Conversions and Statistical Analysis:

All samples were analyzed colorimetrically for NH_4^+ and NO_3^- using the indophenol blue and salicylic acid methods respectively (Keeney and Nelson 1982). Laboratory analysis yielded values for mineral N concentration in sample solutions. Lysimeter results are

presented directly as mineral N concentration in sample solution. IEM results were converted to the quantity of N extracted from membranes by the product of extractant mineral N concentration and extractant volume used (20 ml). The quantity of mineral N extracted from IEMs was expressed per unit area of membrane by dividing by the membrane area (13.6 cm^2). Regressions between maize grain yield and cumulative N concentration in lysimeters, and cumulative N flux to IEMs were conducted using Microsoft Excel 7.0 software. Analysis of variance and mean separations for treatment crop yield were conducted by J.C. Alegre using SAS software.

3.3 Results

Rainfall, soil moisture content and potential leaching:

Rainfall was quite well distributed on a weekly basis throughout the sampling period, with the exception of weeks 5 and 7 (figure 3.4). Soil moisture content was very stable although samples were not well distributed through the measurement period (figure 3.5). Water retention curves (figure 3.1) suggest that gravimetric field capacity is between 18 and 21 percent in the upper 30 cm layer. The potential maximum infiltration of cumulative precipitation into the soil under piston-displacement flow was calculated for the period following the burn (Table 3.2). This calculation assumes a) piston-displacement transfer of water through the soil (Seyfried and Rao 1991) and b) 0 evapotranspiration and the soil always at field capacity (thus net drainage is equal to precipitation) (see Appendix 4). Consistent precipitation in the first 4 weeks after burning allowed rapid, potentially deep transfers of added precipitation into the soil.

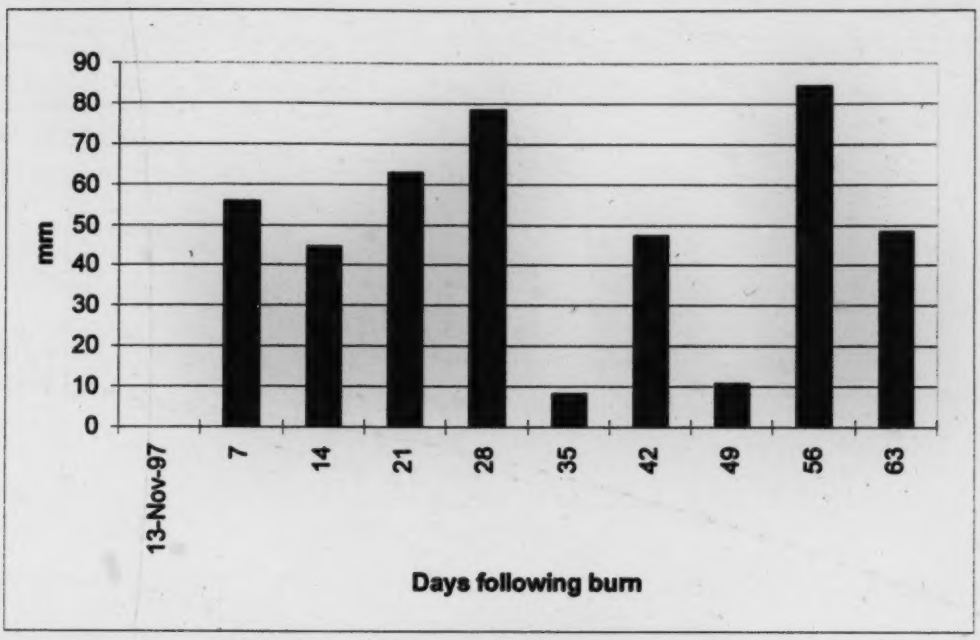

Figure 3.4 Weekly rainfall in the 9 weeks following slash and burn.

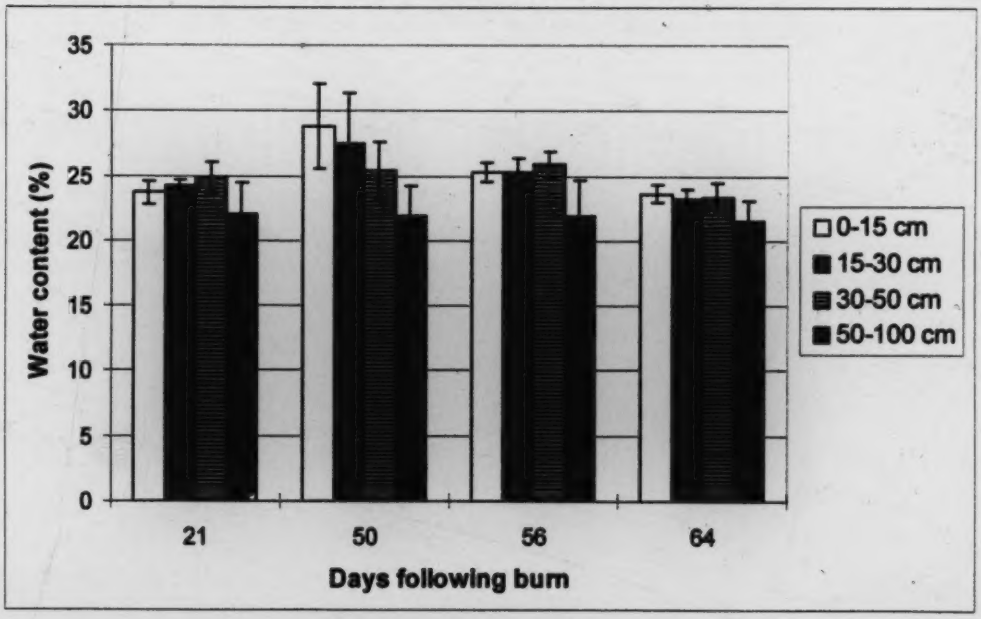

Figure 3.5 Gravimetric soil moisture content at selected depths on 4 dates following the burn. Gravimetric field capacity (0.033 Mpa) ≅ 20%.

Table 3.2 Potential maximum infiltration of precipitation following burn.

Days following burn	Cumulative Rainfall (mm)	Infiltration (mm) †
11	81.6	291
17	128.3	470
21	163.2	589
27	241.1	852
34	249.9	877
40	297.3	1032
54	357.9	1234
57	400.2	1371
64	445.6	1516

† Calculation assumes soil is always at field capacity, no evapotranspiration, and piston-displacement transfer of water. See Appendix 4.

Suction lysimeter sampling:

Variability in precipitation was not reflected in mineral N concentrations in soil solution (Figure 3.6). There are a number of patterns notable in the soils of all or most of the treatments examined. Mineral N concentrations at 15 cm, 30 cm and 50 cm drop after day 40, while concentrations at 100 cm were stable or rising after day 40. Day 40 was the most common peak in N concentration at the 15 cm and 30 cm depths (Figures 3.8 - 3.13). At the end of the measurement period, N concentrations at 15 cm (rooting zone ≅ 0-20 cm) were equal to and usually less than concentrations at deeper depths. The ratio of mineral N at 15 cm: 50 cm declines consistently from 2-5 to below 1 for all treatments (Figure 3.7). At depths of 50 and 100 cm, peaks or sharp increases in N concentrations are simultaneous to, or slightly lagged from, increases or peaks at 15 and 30 cm (Figures 3.8 - 3.13).

Some patterns in mineral N distribution were specific to fallow treatments. In general, mineral N concentrations appeared lower in the natural fallow (Figure 3.8) than in the other treatments (Figures 3.9 - 3.13). In *Inga* treatments (pure *Inga* and *Inga/Centrosema* mix) appeared to have the highest concentrations of N in soil solution and the greatest inter-depth differences. Also, mineral N concentrations at 100 cm in *Inga* treatments tended to increase markedly throughout the measurement period (Figures 3.9 and 3.10).

Especially where *Centrosema* was present (*Inga/Centrosema* mix, *Colubrina/Centrosema* mix, *Centrosema* pure), a bi-modal pattern of N concentration occurred at 15 cm, 30 cm, or both 15 cm and 30 cm (Figures 3.10, 3.12, 3.13). The patterns of N concentration are remarkably similar when *Colubrina/ Centrosema* and pure *Centrosema* treatments are compared (Figures 3.12 and 3.13).

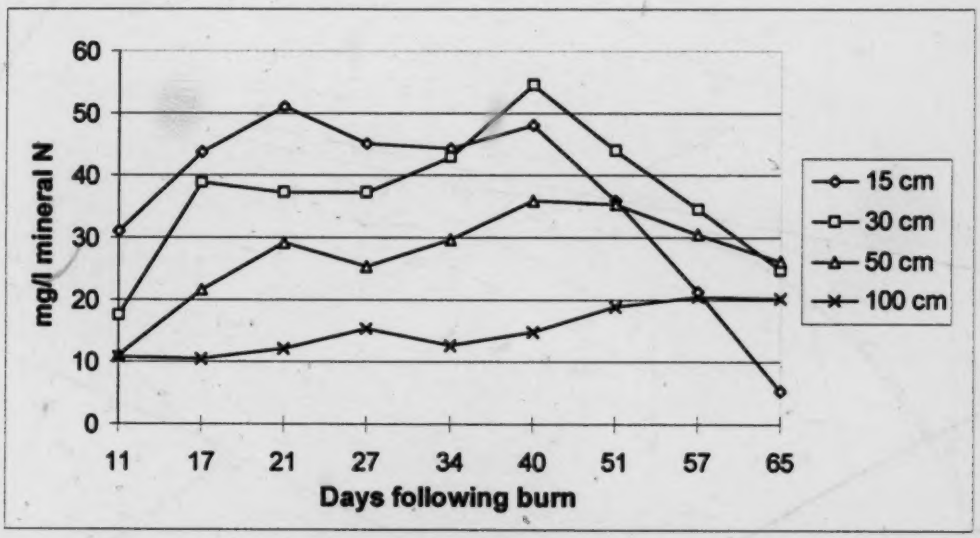

Figure 3.6 Concentration of mineral N (mg/l) following slash and burn disturbance.

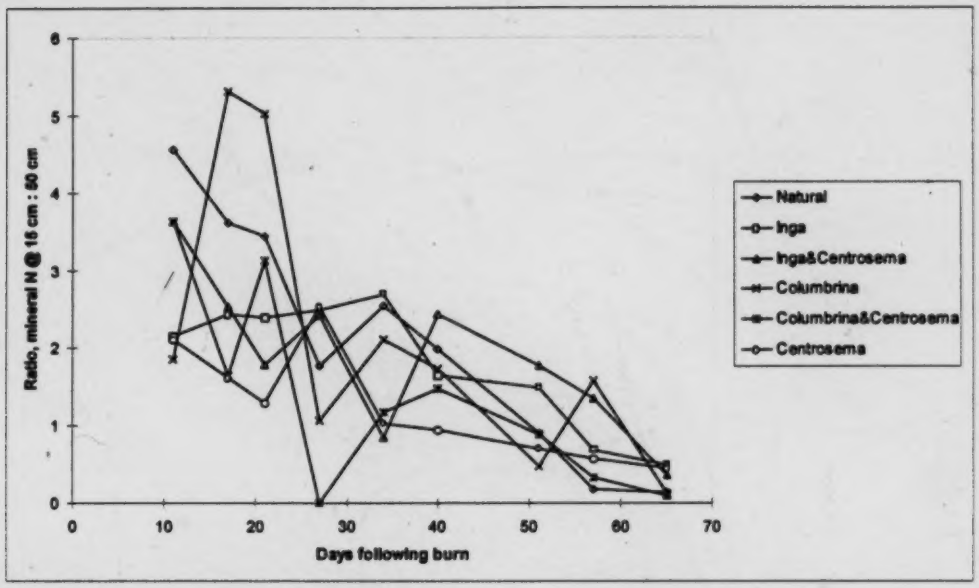

Figure 3.7 Ratio of mineral N at 15 cm : mineral N at 50 cm after burning fallow vegetation and planting to maize.

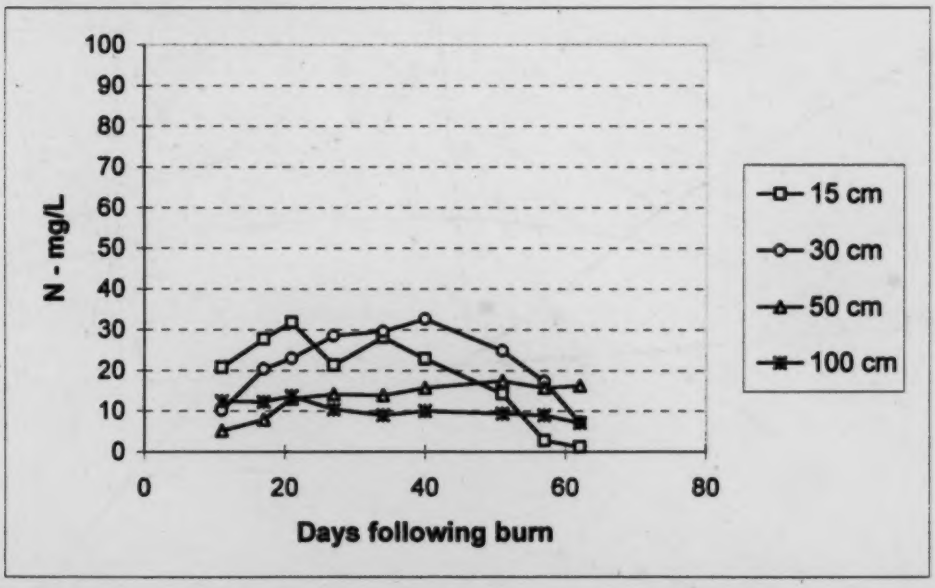

Figure 3.8 Natural treatment after burning fallow vegetation and planting to maize. NH_4^+ plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.

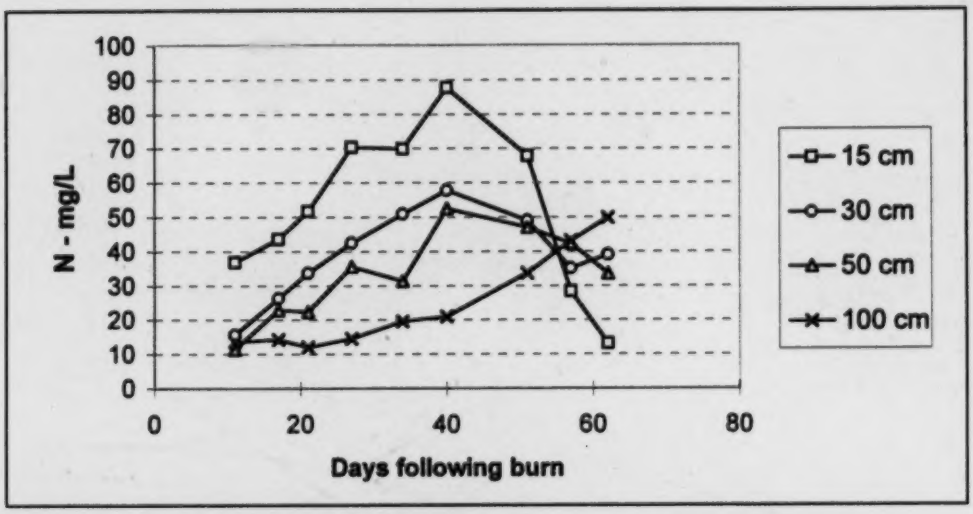

Figure 3.9 ***Inga*** **treatment after burning fallow vegetation and planting to maize. NH_4^+ plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.**

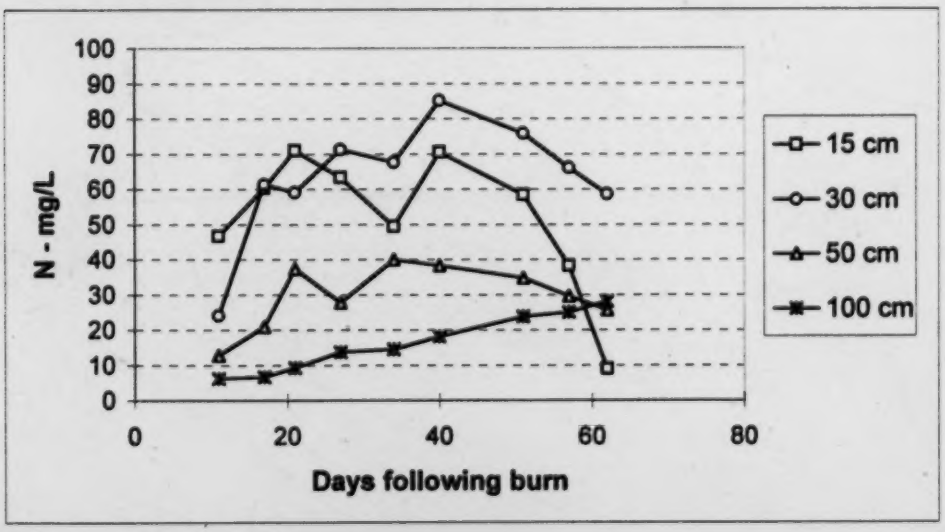

Figure 3.10 ***Inga/Centrosema*** **treatment after burning fallow vegetation and planting to maize. NH_4^+ plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.**

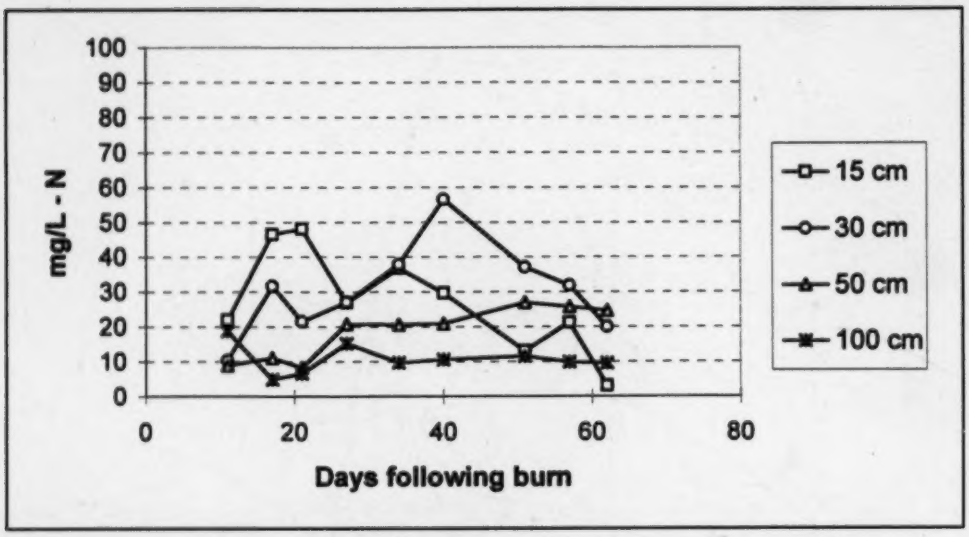

Figure 3.11 ***Colubrina*** **treatment after burning fallow vegetation and planting to maize. NH_4^+ plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.**

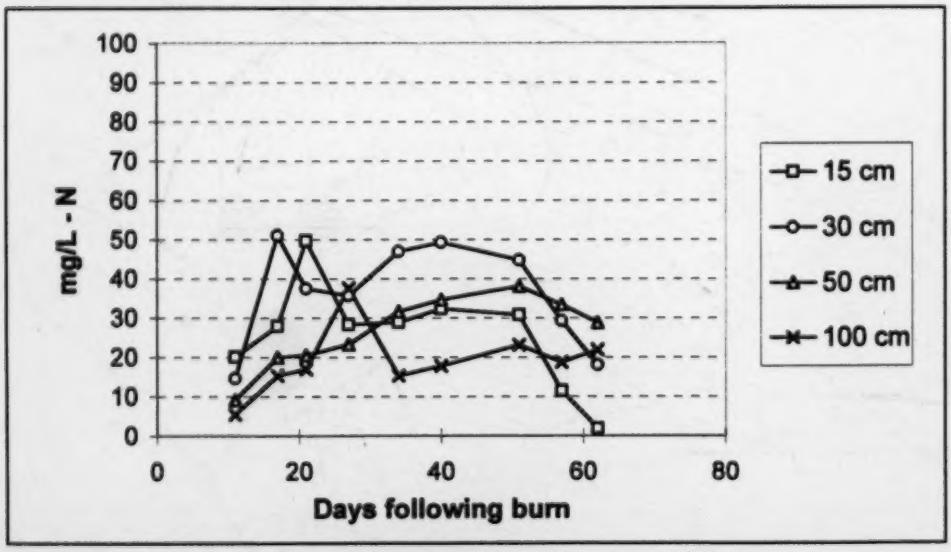

Figure 3.12 ***Colubrina/Centrosema*** **treatment after burning fallow vegetation and planting to maize. NH_4^+plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.**

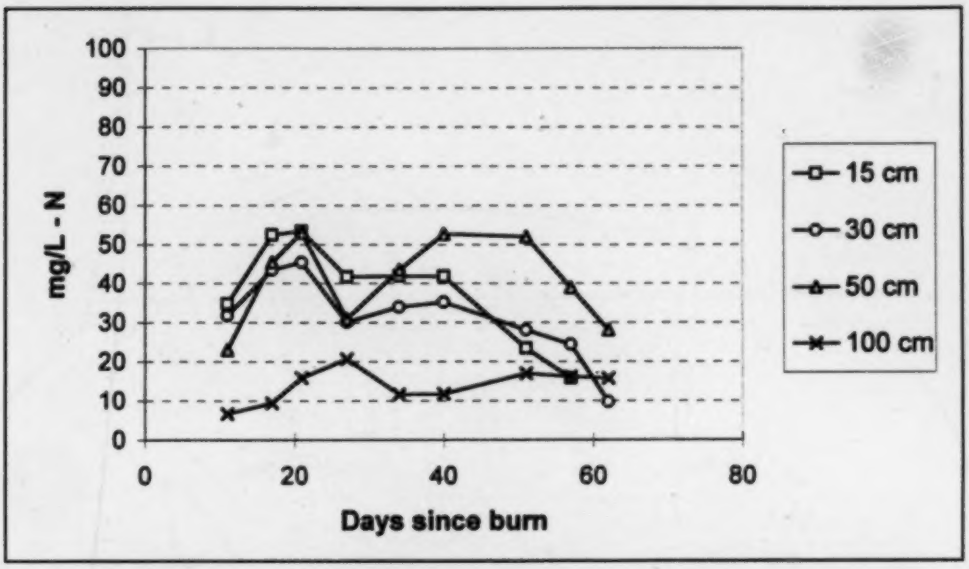

Figure 3.13 *Centrosema* treatment after burning fallow vegetation and planting to maize. NH_4^+ plus NO_3^- concentrations in soil solution samples extracted by porous cup lysimeters installed at sequential depths.

The proportion of soil solution mineral N (NO_3^- + NH_4^+) present as NH_4^+ dropped rapidly in the 3 weeks immediately after the burn (Figures 3.14 -3.19). This trend was common to all treatments and at all soil depths examined. Results from chapter 2 and previous site data suggest that NH_4^+ is normally less than 10% of the mineral N in soil solution either in the rooting zone or deeper layers (see Chapter 2, and Figures 3.2 and 3.3 above).

In soil of the pure *Inga* fallow, NH_4^+ concentrations dropped less rapidly, and there were more inter-depth differences (Figure 3.15), in contrast to the other treatments. As well, NH_4^+ proportions at 100 cm depth were initially quite high (almost 70%) and dropped very rapidly after day 17 (*Inga* fallow soil). In the soil of the *Centrosema* fallow, the proportion of NH_4^+ at the first measurement date was 10% - 15% lower than the other treatments at each respective depth (Figure 3.19).

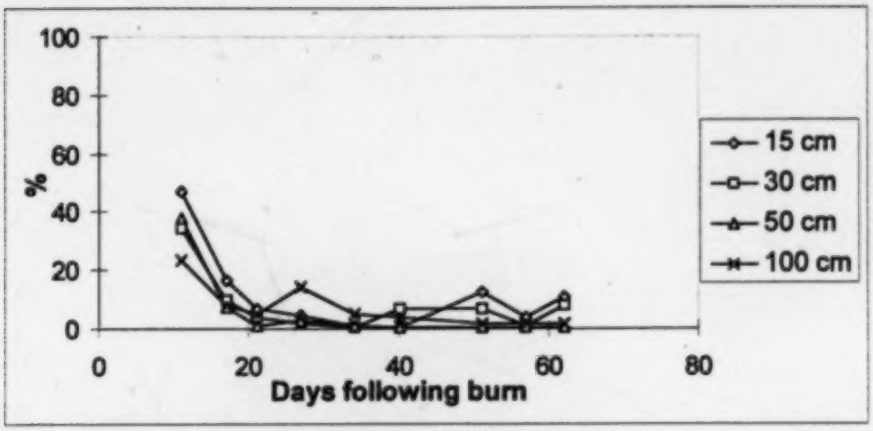

Figure 3.14 Natural treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.

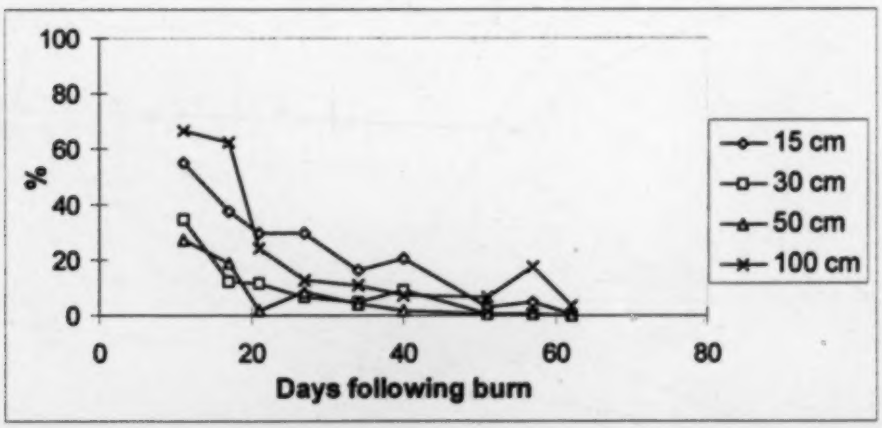

Figure 3.15 *Inga* treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.

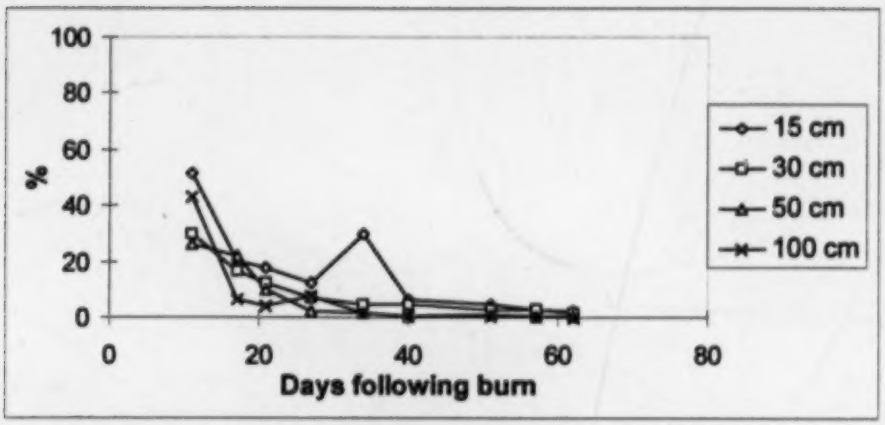

Figure 3.16 ***Inga & Centrosema*** **treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.**

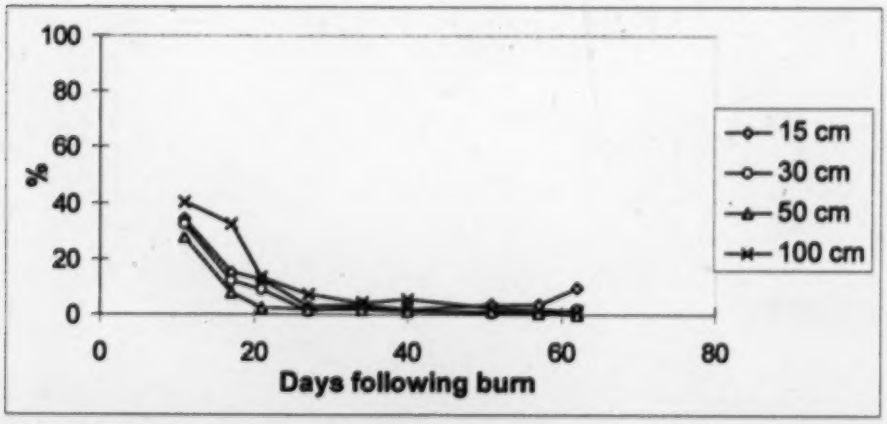

Figure 3.17 ***Colubrina*** **treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.**

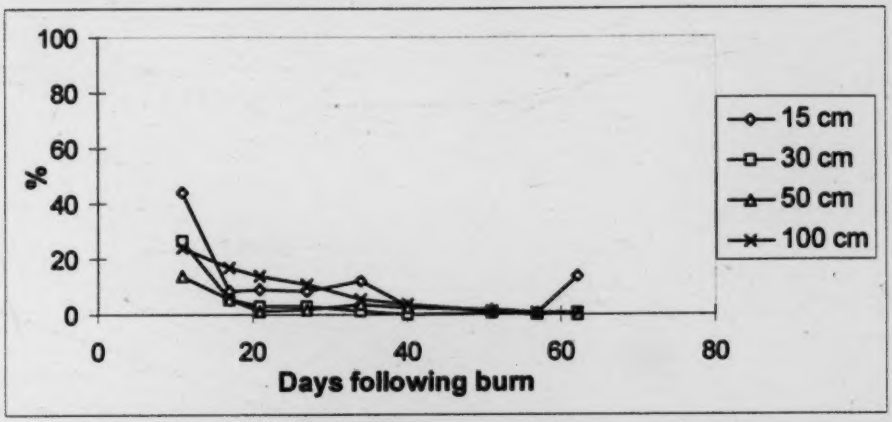

Figure 3.18 ***Colubrina & Centrosema*** **treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.**

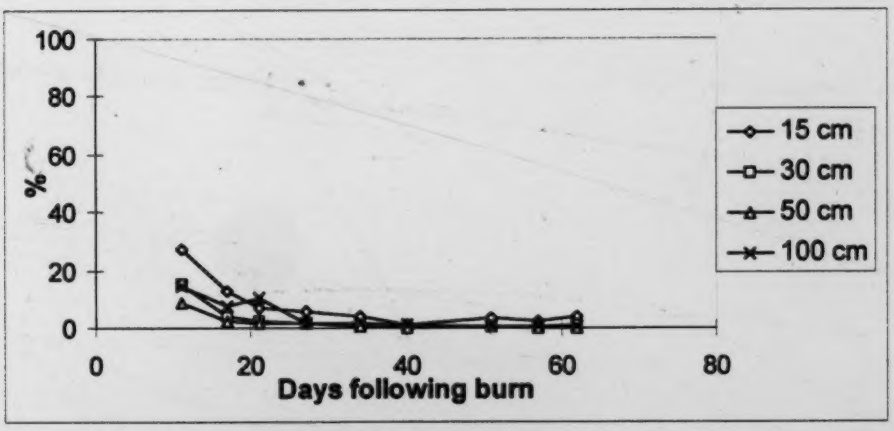

Figure 3.19 ***Centrosema*** **treatment after burning fallow vegetation and planting to maize. Percent of sampled mineral N as NH_4^+.**

N flux measured by ion exchange membranes:

Ion exchange membrane (IEM) data was collected for the Natural, *Inga*, *Colubrina* and *Centrosema* fallow soils at 2 and 15 cm depths (Figures 3.20 -3.23). The action of some

soil animal, likely a type of termite, made holes in the membranes. By the end of the experiment, some membranes had to be removed from service and virtually all had holes. Membranes were subjectively removed from service when more than about 10% of their area was gone. As well as masticating membrane fabric, the termintes occaisionally built a hard soil crust against the membrane. The amount of membrane available for cation exchange was reduced, and the formation of a soil-crust against the membrane probably altered the rate of diffusion of ions to the membrane or altered the concentration of ammonium near the membrane. However, termite interference did not result in erratic NH_4^+ values in the membrane extracts. Values from membranes with interference by termite activity were always in the same order of magnitude and similar to values from replicates without interference. No attempt was made to adjust IEM results for reduction of membrane area.

At 2 cm depth, the pattern of mineral N flux to membranes was remarkably similar over time through all treatments (Figures 3.20-3.23). For the period between 6 and 13 days after the burn, total mineral N flux to membranes was between 0.5 and 1.0 micrograms per cm^2 for all treatments. Mineral N flux in all treatments increased slightly during the period 19-26 days and then dropped to or below the 0.5 microgram/cm^2 level for the periods 33-40 and 47-54 days. Variability in mineral N flux, as indicated by bars of standard deviation, was quite low through all treatments at 2 cm depth.

At 15 cm depth the patterns of mineral N flux were also similar between treatments, with the possible exception of the *Centrosema* fallow soil. Mineral N flux at 15 cm depth was relatively low during the initial measurement period, increased during the period 19-26 days and remained stable or declined slightly during the last two periods (Figures 2.20-2.23). In the cases of Natural, *Inga*, and *Colubrina*, N flux during the last three measurement periods was essentially constant, while flux in the *Centrosema* treatment dropped in the last two periods.

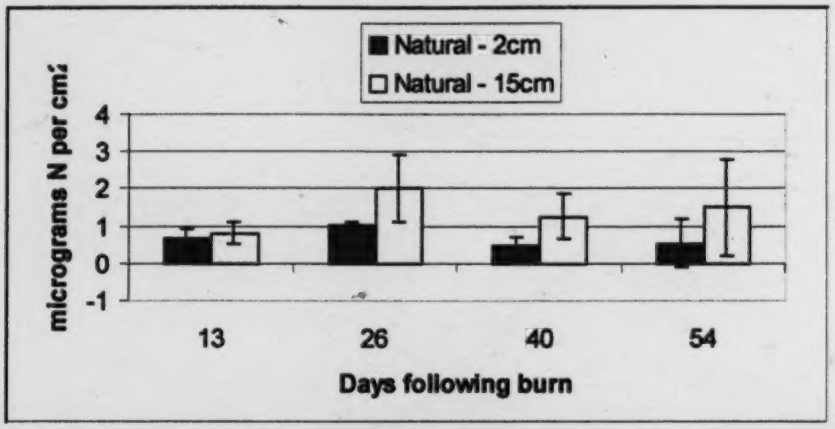

Figure 3.20 Natural treatment after burning fallow vegetation and planting to maize. Micrograms of NH_4^+ and NO_3^- flux per cm^2 of ion exchange membrane; each sample date represents the end of 7 days exposure.

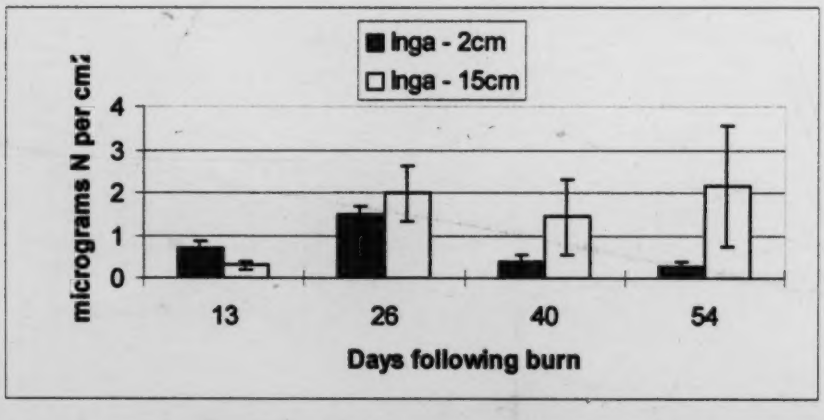

Figure 3.21 *Inga* treatment after burning fallow vegetation and planting to maize. Micrograms of NH_4^+ and NO_3^- flux per cm^2 of ion exchange membrane; each sample date represents the end of 7 days exposure.

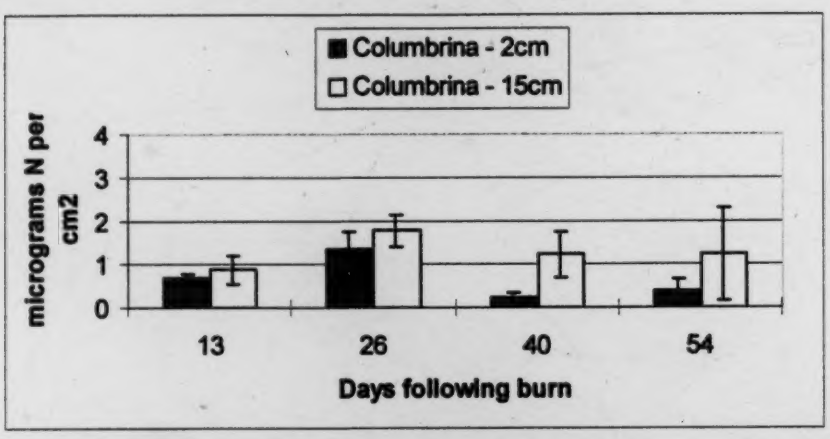

Figure 3.22 ***Colubrina*** **treatment after burning fallow vegetation and planting to maize. Micrograms of NH_4^+ and NO_3^- flux per cm^2 of ion exchange membrane; each sample date represents the end of 7 days exposure.**

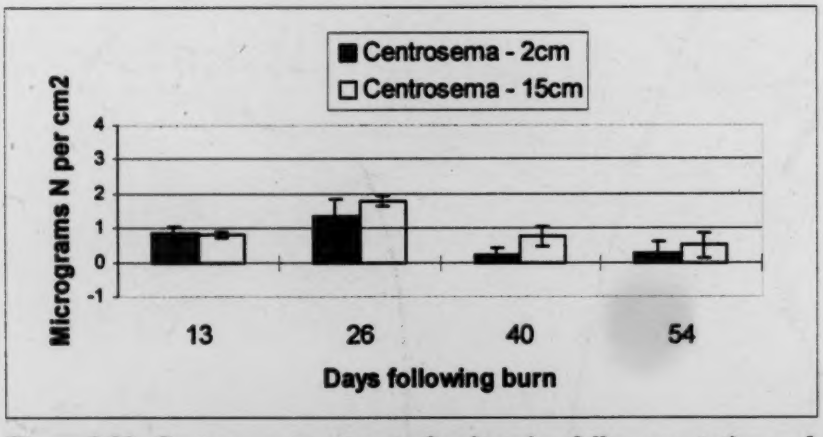

Figure 3.23 ***Centrosema*** **treatment after burning fallow vegetation and planting to maize. Micrograms of NH_4^+ and NO_3^- flux per cm^2 of ion exchange membrane; each sample date represents the end of 7 days exposure.**

In general the proportion of mineral N flux to IEMs as NH_4^+ declined with time (Figures 3.24 - 3.27). At 2 cm there was always more NH_4^+ flux than at 15 cm. In the Natural fallow soil (Figure 3.24) the decline in NH_4^+ flux was not prominent at 2 cm. In the *Colubrina* fallow soil, there was no decline in NH_4^+ flux at 15 cm, and in the *Centrosema* fallow soil NH_4^+ flux was always small, especially at 15 cm.

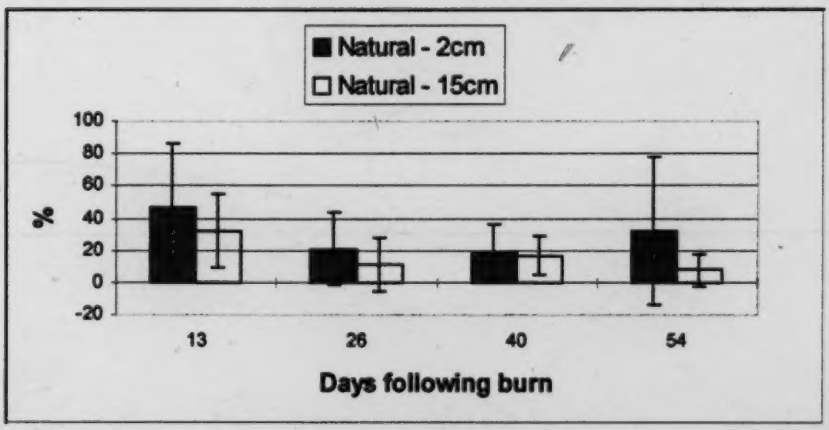

Figure 3.24 Natural treatment after burning fallow vegetation and planting to maize. % of mineral N (NH_4^+ and NO_3^-) flux to ion exchange membranes as NH_4^+.

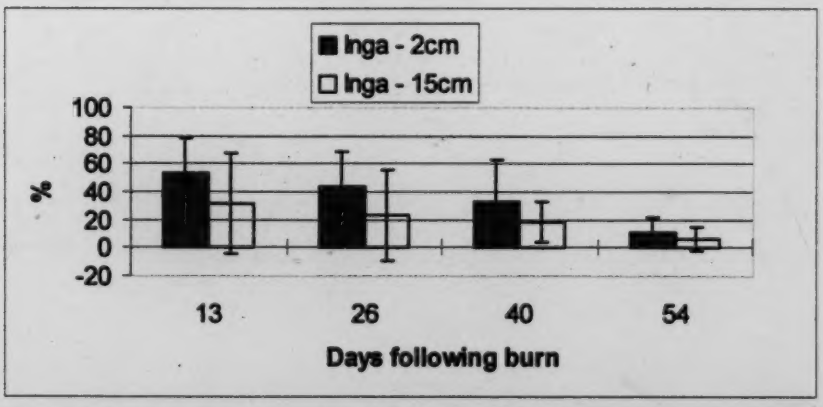

Figure 3.25 *Inga* treatment after burning fallow vegetation and planting to maize. % of mineral N (NH_4^+ and NO_3^-) flux to ion exchange membranes as NH_4^+.

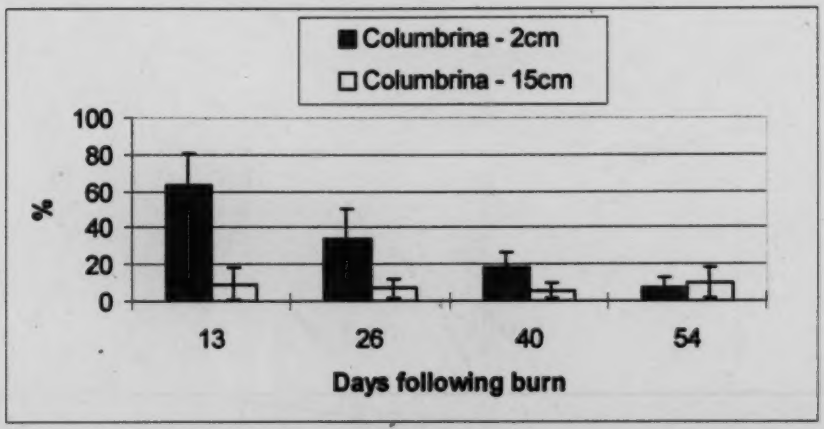

Figure 3.26 ***Colubrina*** **treatment after burning fallow vegetation and planting to maize. % of mineral N (NH_4^+ and NO_3^-) flux to ion exchange membranes as NH_4^+.**

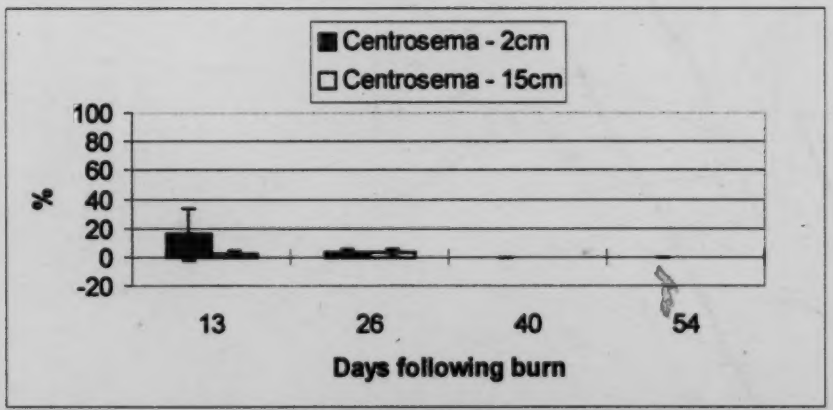

Figure 3.27 ***Centrosema*** **treatment after burning fallow vegetation and planting to maize. % of mineral N (NH_4^+ and NO_3^-) flux to ion exchange membranes as NH_4^+.**

Comparison of patterns in IEM vs lysimeter data and relationship to yields:

In the soils of all fallow treatments, IEMs and suction lysimeters both reflected increasing mineral N concentrations in the first 3 weeks following the broadcast burn. At later measurement dates, however, results from suction lysimeters in *Colubrina* and Natural fallow soils suggest dropping N concentrations while results from IEMs do not. In the *Centrosema* fallow soil, the pattern of mineral N concentration sampled by suction lysimeters is in general similar to the pattern of N flux to IEMs over time. Note that different time scales were involved in sampling: lysimeter samples were collected over a 12 hour period, and IEMs were allowed one week to collect mineral N.

Maize yields achieved previously at the San Ramon experiment station under "complete" mineral fertilization were between 3 and 4 tonnes per hectare (Sanchez et al. 1982). In comparison, maize yields under improved fallow were low. There were some significant differences between treatments (Table 3.3). Mineral N measurements made in the rooting zone by either suction lysimeters or IEMs were not closely related to the yields in respective treatments (Table3.3). Although yields suggest a treatment response from *Centrosema* fallow, all of the treatments had similar amounts of mineral N in the soil as measured by IEM. For example, the natural fallow soil had similar or greater amounts of IEM-N than the *Centrosema* fallow soil, yet the Natural fallow only produced 54 % of the yield of *Centrosema*. Suction Lysimeters suggest that *Inga* and *Inga* + *Centrosema* had distinctly more N at 15 cm depth than the other fallow treatments but this is not reflected in the maize yields observed.

Table 3.3 Grain yield and cumulative N measurements in the 0-15 cm zone

Treatment	Grain Yield (kg ha^{-1})	Cumulative N in lysimeters @ 15cm (mg/L)	Cumulative N flux to IEM @ 2cm (ug/cm^2)	Cumulative N flux to IEM @ 15cm (ug/cm^2)
Centrosema (CM)	926 a	310.9	2.77	3.88
CM + *Colubrina*	867 a b	231.6		
Inga	577 a b	469.6	2.86	5.88
Inga + CM	502 b	467.2		
Natural Fallow	498 b	170.9	2.76	5.58
Colubrina	484 b	247.9	2.71	5.11
Correlation (r) with Grain Yield		-0.16	0.14	-0.85

Means separated by LSD = (390)
Means followed by the same letter are not significantly different (P≤0.05).

Table 3.4. Correlation (r) between lysimeter data (mg/L at 15 cm) and IEM data (ug/cm^2 @ 15 cm).

IEM sample days	6-13	19-26	19-26	19-26	33-40	33-40	47-54	47-54
Lysimeter sample day	11	17	21	27	34	40	51	57
Coefficient (r) †	-0.14	-0.50	-0.52	-0.16	0.17	0.32	0.20	0.38

† None of the regressions is significant (P≤0.05).

3.4 Discussion

Mineral N increase and NH4+ flush as proportion of mineral N:

Increased concentration of post-fire soil mineral N in the rooting zone is commonly reported following fire in a range of ecosystems (Christensen 1973; Seubert et al. 1977; Hobbs and Schimel 1983; Klopatek *et al.* 1990; Singh 1994; Weston and Attiwill 1996).

There is little agreement in the literature, however, on the mechanisms which are responsible for mineral N increases. Possible mechanisms include: 1) increased mineralization due to soil temperature and pH increase, 2) mineralization of heat-killed microbial biomass, 3) de-amination of organo-mineral complexes and NH_4^+ release from clay inter-layers due to heating, 4) mineralization of fine roots, 5) curtailed plant N uptake, and 6) N deposition as a constituent of ash. Mechanisms could be site- and event-specific, because of variability both in soil-plant systems and in the conditions imparted by fires.

Ash formation and deposition alter soil chemical and physical characteristics and conditions for microbial activity. Raison (1979) summarizes that pH increase, addition of organic substrate, and increased concentrations of mineral nutrients are fire processes which can alter microbial activity. Mineral cations in ash rapidly transform from oxides to carbonates, both of which are alkaline in reaction (Kimmins 1987). Increases in pH may stimulate microbial activity (Woodmansee and Wallach 1981; Grasso et al. 1996) and in particular nitrifiers which are normally limited by low pH (Kimmins 1987). Heating during burning kills some of the microbial population, but re-colonization is usually rapid (Grasso *et al.* 1996). In the acid soils of the Upper Amazon basin, pH increase following ash addition may be one of the most important fertility benefits from burning (J.C. Alegre - personal communication). While soils of the Amazon are warm, vegetation removal and lowered albedo could result in soil temperature increases which also stimulate microbial activity. If, however, increased microbial activity was responsible for the rapid mineral N increases reported here, then available substrates had to have C/N ratios which allowed immediate net mineralization.

Physical and chemical transformations of organic matter during burning could have contributed to mineral N increases after the burn. Fires under field conditions often leave incompletely burned organic matter: black ash contains residual organic matter while white does not (Raison 1979). Prieto-Fernandez et al. (1993) found that total organic N

of the 0-5 cm layer in a Humic Cambisol was increased significantly one month following burning. They suggest that the increase was due to residual organic matter in ash, presumably transferred to the soil as small particles following the burn. Blank and Zamudio (1998) (citing Dunn et al. 1979 and Debano et al. 1979) suggest that incompletely burned organic matter is predisposed to mineralization. Formation of char is one pathway of pyrolytic degradation (Tillman et al. 1981), but it is not clear whether amino groups are retained, and if so whether they are susceptible to biological degradation. Charcoal constitutes one of the most slowly decomposed fractions of soil organic matter, however, so it may not be a labile N source (W.B. McGill - personal communication). As noted earlier, it appears that little mineral N is retained in ash after burning.

In humid tropical forests, nutrients are normally very effectively recycled by vegetation (Lodge et al. 1994). Upon slashing and burning of the fallow vegetation, then, root systems die and nutrient uptake is curtailed, but additions of root litter and activity of microorganisms could increase. Note that much larger fluxes of mineral N were evident on this site during the first months of vegetation establishment (October 1994 to January 1995) (Figures 3.2 and 3.3). Slashing and burning also resulted in addition of freshly killed and non-burned organic matter to the soil surface where it was mineralized (eg. *Centrosema)*. As well as incomplete burning at the surface, the death of root systems and rapid decomposition of fine roots probably contributes to increased mineral N concentrations when vegetation is killed (Woodmansee and Wallach 1981). Seubert et al. (1977) found high concentrations of soil mineral N following both bulldozer clearing and slash and burn at Yurimaguas, and they suggest that decomposing roots (common to both types of disturbance), was the major contribution to this mineral N flush.

As well as increased total mineral N, an increase in the proportion of mineral N as NH_4^+ is commonly reported in the literature (Christensen 1973; Klopatek et al. 1990; Prieto-Fernandez 1993; Blank et al. 1996). Blank et al. (1996) suggest that increases in

ammonifying microbes coupled with suppression of nitrifier populations and concurrent NH_4^+ release from soil organic matter or clay interlayers is responsible for the NH_4^+ flush. Alternatively, ash induced pH increase could stimulate nitrifiers as noted earlier. Woodmansee and Wallach (1981) point out that nitrification is usually limited by rapid plant uptake of NH_4^+ where established root systems exist. Thus the post-burn NH_4^+ flush reported here could be attributed to curtailed ammonium uptake after root death followed by gradual response to available NH_4^+ by nitrifiers. Indeed, NH_4^+ concentrations in this experiment were relatively high following the burn but dropped and stabilized within one month.

Bi-modal N flows were characteristic of treatments including *Centrosema*, where burning of organic matter was incomplete. The sharp initial peak is consistent with the mineral N flux throughout the experiment, while the second period of mineral N mobilization occurred 40 - 50 days following the burn. The second period of high mineral N concentrations is perhaps consistent with biologic decomposition of the unburned layer. Note that crop yields were highest in treatments which included *Centrosema*, although this could have been due to a soil chemical or physical property imparted by *Centrosema* other than the pattern of N flow.

N decline in maize rooting zone and deep N loss:

Reduced mineral N concentrations in the rooting zone 6 weeks after the burn was likely due to both leaching transfer to deeper depths (note increasing N concentrations of deep layers) and immobilization of mineral N in the maize crop. The concentration declines are most marked at 15 cm depth and correspond to the progressive development of the maize crop.

The possible mechanisms for an NH_4^+ increase are phenomena which happen near the soil surface at this site. Ash is deposited at the soil surface and soil heating during burning of either forest slash or grasslands is limited to the upper few cm (Raison 1979).

Decomposing roots are located near the surface, as rooting in acid soils is restricted to upper layers due to Al^{3+} and Mn^{2+} toxicity or acid induced Ca deficiency (Fisher and Juo 1995). The finding of high concentrations of NH_4^+ simultaneously at all depths following burning, therefore, suggests that ammonium was quickly leached to 50 and 100 cm depths from the surface layers. Simultaneous or slightly lagged peaks in total mineral N concentration at lower soil layers compared to upper layers is further evidence that mineral N was quickly leached to deep layers following burning.

The rooting zone of maize in acid soils of the region is not greater than 20 cm (JC Alegre - personal communication). Thus mineral N below 20 cm on this site was likely unavailable during cropping and unrecoverable barring rapid establishment of a deep rooting species. As well as evidence of immediate N transfers to depth, there were gradual increases in mineral N concentration at either 50 or 100 cm in virtually every treatment. Mineral N at these depths remains susceptible to leaching if it is not somehow immobilized. Note that in both treatments which included *Centrosema*, there were early peaks in total mineral N at 100 cm, suggesting that this N may have accumulated temporarily at 100 cm and then was leached to deeper layers. Thus mineral N loss from the crop rooting zone probably happened immediately during saturated flow following heavy rain. Gradual accumulation at deep layers was too deep for crop recovery and remained susceptible to yet deeper transfers.

The increase in mineral N at 100 cm depth was most prominent in the soil of the *Inga* fallow. In general N concentrations were highest in this soil, but *Inga* trees may also have left many root channels enhancing transfer of mineral N following death of the tree.

Utility of IEMs vs lysimeters, and correlation with maize yield:

Maize yield was a function of growing conditions including light, temperature and humidity, water and nutrient availability. Only N data were collected in this experiment and although N is commonly limiting crop yields in this region (Sanchez 1976),

deficiencies of other nutrients or growth factors are also important. It is possible that calcium and phosphorus deficiencies also limited yield in this experiment. With the exception of some plots of the Natural and *Colubrina* treatments, height growth was vigorous.

IEM and lysimeter results were not closely related to maize yields, and since only mineral N was measured, it is difficult to comment on the usefulness of either in assessment of soil fertility. Relative to the lysimeters, IEMs recorded relatively higher amounts of mineral N at 15 cm during late stages. IEMs rely on diffusion of mineral ions to the membrane surface. Course textures and dry soils could limit diffusion rates and large aggregates may limit soil contact with the membrane, but these limitations are not consistent with relatively high concentrations of N flux to the membranes at late stages.

The tension applied to lysimeters extracted a liquid sample which had been held by the soil at lesser tension. A fraction of soil water held tightly and very close to mineral surfaces was not extracted. Ion concentrations may be higher near mineral surfaces; these ions might have diffused to IEMs while not present in lysimeter samples. The trend of NH_4^+ decline following the burn is more prominent in lysimeter results than IEM results. Relative to lysimeters at 15 cm, IEMs at 15 cm appeared to measure smaller amounts of NH_4^+ the 7-13 day period after the burn; this is apparent in all treatments. IEMs were installed for a period of one week and sampling was not coordinated with precipitation, therefore NH_4^+ near membranes may have been nitrified before diffusing to membrane surfaces, or NH_4^+ diffusion could have been slower than NO_3^- diffusion. Lysimeter sample collection was restricted to a short period following moderate to heavy precipitation events allowing little time for nitrification of NH_4^+ newly transported in leachate. It is not likely that the NH_4^+ flush was an artifact resulting from the sand-slurry used to install the lysimeters, because this material yielded negligible concentrations of NH_4^+ when artificially leached in the laboratory (data not shown).

3.5 Conclusions

A mineral N flush occurred in association with vegetation disturbance in this improved fallow system. Some of this mineral N appeared to be rapidly leached to deep soil layers (50 cm +) as indicated by the appearance of NH_4^+ at these depths, simultaneous peaks in mineral N concentration at upper and deep soil layers, and gradually increasing mineral N at 100 cm. The calculated infiltration of the leaching front suggests that mineral N could have been transferred below the maize rooting zone in the first 17 days after the burn, possibly at the cost of crop yield.

IEMs produced patterns of mineral N flux that did not correspond to patterns of mineral N concentration in suction lysimeter samples. Neither of these techniques for measurement of soil mineral N status were correlated to grain yields; yields may have been constrained by nutrient deficiencies other than N.

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4. N mineralization from prunings incubated using a column-leaching technique

4.1 Introduction

Synchrony between nutrient release from organic fertilizers and nutrient uptake by crops has recently been the focus of research in tropical ecosystems. Achieving synchrony requires that plant nutrient uptake be matched with periods of most rapid mineralization; annual crops take up nutrients only on a seasonal basis. Characterization of nitrogen (N) release from tropical legumes reveals that lignin (L) and polyphenols (PP) may control rates of N mineralization when C:N ratios are low enough to allow immediate mineralization. Thus even when high-N sources of plant N are available for fertilizer, their lignin and polyphenolic contents, and their pattern of N release, must be considered.

In inter-species comparison of tropical plant materials, group ranking in L+PP:N ratio is commonly highly correlated with group ranking in rate of N release (Fox et al. 1990; Constantinides and Fownes 1994; Handayanto et al. 1994 and 1997; Lehmann et al. 1995). In a similar manner, chemical indices such as PP:N or C:N are also correlated with mineralization rates in other investigations (Mellilo et al. 1982; Palm and Sanchez 1990 and 1991). However, simple correlation between chemical characteristics of prunings and cumulative N release after a fixed time is cumbersome because results depend upon the length of each particular experiment. Precise use of agroforestry prunings will be aided by relating chemical characteristics to the patterns of mineralization rates over time; mathematical mineralization models may expedite this exercise.

The capacity of some polyphenols to bind proteins into protein-phenol complexes is proposed as a mechanism that reduces mineralization rates; binding may limit substrate availability and deactivate extra-cellular enzymes. Polyphenols can also be toxic to microorganisms thus reducing mineralization. An understanding of phenolic controls on mineralization rates will be required if fertility benefits from prunings is to be maximized.

Northup et al. (1995) suggest that high litter phenolic concentrations favor the formation of dissolved organic nitrogen (DON) rather than ammonium during decomposition. The capacity of phenolics to bind proteins, conceivably forming DON, was closely related to reduced mineralization rates and mulch N recovery by maize crops (Handayanto et al. 1994; 1997). Many types of microbes may lack the ability to degrade DON in protein-tannin complexes (Bending and Read 1996); DON may be accessible primarily through mycorrhizal root associations. Further, DON is probably less susceptible to leaching in soils than is mineral N (Fayey et al. 1985). DON formation from agroforestry materials and its management for soil fertility has not been directly investigated.

Mixed portions of materials high and low in polyphenols may be used to manage N release (Handayanto et al. 1997). Although the potential of mixing materials has been discussed, reliable prediction of mix behavior has not been achieved. Swift (1987) hypothesized that a mix of high and low quality materials would result in reduced initial release followed by increased late release. Palm (1995) suggests that mixes generally release nutrients more rapidly than their non-interaction mean. Handayanto et al. (1997) found that phenolics from *Peltophorum dasyrrachis* prunings bound with proteins from *Gliricidia sepium* and reduced mineralization rates when the two were combined in mixture; they suggest that interaction between mix components will occur when either soluble C or protein-binding phenolics move from one component to another.

A number of investigators have fit first order exponential mineralization models to cumulative results from incubating agroforestry materials (Palm and Sanchez 1990 and 1991, Tian et al. 1992, and Handayanto et al. 1994 and 1997). Palm and Sanchez (1990 and 1991) and Handayanto et al. (1994 and 1997) applied the form $Y=e^{(-kt)}$. Tian et al. (1994) included an expression for N_o using the formula $Y=No*e^{(-kt)}$. These models represent the amount of mineralizable N, or the proportions of mineralizable N remaining after time t. The amount mineralized = N_o-Y; or the proportion mineralized = 1-Y. Palm

and Sanchez (1990) comment that the first order model fit their data well but the others do not discuss model fit.

More complex mineralization models such as the Gompertz model can also be fitted to mineralization data. The Gompertz model has the advantage of allowing a more complex (sigmoidal) mineralization curve compared to the exponential model, which assumes that mineralization rates are always highest at the beginning of decomposition. The single exponential form has the advantage of simplicity: complex N mineralization curves can be summarized by a single value (k). Mathematical models such as the Gompertz or exponential allow mineralization parameters to be related to litter chemical characteristics, potentially yielding insights into mechanisms controlling mineralization.

Cumulative data appear to be most commonly used for model fitting. However, Ellert and Bettany (1988) point out potential problems with fitting equations to cumulative mineralization results. First, the use of iterative least squares techniques to find best-fit parameters implies that samples points are independent of each other and have the same inherent uncertainty (or error is independently distributed); this assumption does not hold for cumulative N mineralization data. Second, fitting the first order equation to cumulative data is sometimes completed using logarithmic transformation which may put disproportionate weight on samples late in the data set.

The primary objective of this experiment was to characterize N release from different prunings and soils by connecting system chemical characteristics with modeled and measured N release to leachate. Four hypotheses were formulated and tested to meet this objective.

- L+PP:N is better correlated with N release than C:N ratio (consistent with results from Yurimaguas, Peru).
- DON is not a significant portion of N in soil leachate at 15 cm depth.

- A pruning mixture of equal proportions *Inga/Centrosema* will exhibit rates of N release below rates that could be predicted assuming no interaction between the components; this interaction between pruning types is consistent with phenolic action.
- A mineralization model (modified Gompertz) which incorporates a lag in time of maximum mineralization rate fits incremental N release better than an exponential model, which describes mineralization dynamics in which the maximum rate is at time 0.

A secondary objective was to employ a leaching-column incubation technique with two soils, one from Yurimaguas, Peru and one from Turrialba, Costa Rica. This technique would allow the use of intact soils and prunings and simulate the field conditions of an agroforestry system.

4.2 Materials and Methods

Soils:

Ultisol soil columns were extracted from the site of the field experiments at Yurimaguas reported in Chapters 2 and 3, and their soil characteristics recorded in chapter 2. The Peruvian soil is classified as a fine-loamy, siliceous, isohypothermic Typic Paleudult (Tyler 1975).

Andisol columns were extracted from a site near the village of San Juan Sur, near Turrialba, Costa Rica. The Costa Rican soil is classified as a fine, mixed, isothermic, Acrudoxic Melanudand (Lopez and Kass 1996). The site at San Juan Sur is positioned on a ridge of old lava flow from the Irazu volcano located approximately 20 km to the north-west. Slopes at the site are variable (15 - 50 %). The soil profile is deep and well developed; highly weathered lava rock forms the subsoil, and surface layers are influenced by recent volcanic ash additions (Lebeuf 1993). Surface soil characteristics are summarized in Tables 4.1 and 4.2.

Table 4.1 Andisol characteristics. CATIE experimental site, San Juan Sur, Costa Rica.

Layer	Db *	P (Olsen) * (mg/g)	CEC * ($cmol^+ kg^{-1}$)	Al Satn.* (%)
0-5 cm	0.63	6.9	2.08	79.7
5-10 cm	0.63	3.2	1.36	79.8
10-15 cm	0.63	2.1	1.11	78.3
Mean	0.63	4.1	1.52	79.3

Source: DCL Kass, personal communication.
* Db = bulk density, P (Olsen) = extractable phosphorus by Olsen method, CEC = cation exchange capacity, Al Satn. = aluminum saturation

Table 4.2 Soil Characteristics of Ultisol in comparison to Andisol

	Andisols				Ultisols			
Layer	Db	pH ††	O.C. † (g/kg)	N † (g/kg)	Db *	pH *	O.C. * (g/kg)	N * (g/kg)
0-5	0.63	4.2	61.4	0.58	1.21	5.3	12.11	1.22
5-10	0.63	4.3	61.4	0.58	1.21	5.2	7.95	0.81
10-15	0.63	4.4	61.4	0.58	1.39	5.2	7.95	0.81
Mean	**0.63**	**4.3**	**61.4**	**0.58**	**1.27**	**5.2**	**9.34**	**0.95**

† Source: Garzon (1991)
†† Source: DCL Kass, personal communication
* Source: JC Alegre, personal communication

Columns of soil were extracted by inserting precut lengths of PVC pipe to a depth of about 18 cm into the soil, gently breaking contact between the soil core and sub-soil, and extracting the intact soil column. PVC pipe for Ultisol and Andisol columns were 10.2 and 10.8 cm inside diameter, respectively. In the greenhouse, soil columns were adjusted to 15 cm depth by shaving away the subsurface portion as required. Columns were maintained inside their original PVC pipe for the duration of the experiment. To minimize variability in soils extracted, columns were taken from a 1-2 m^2 area at each site. In

Yurimaguas, columns were taken from a plot which was fallowed (3 years) under *Centrosema macrocarpum* and had a mature maize crop at the time of sampling. Yurimaguas columns were dried at 60° to 80-90 % of their original wet weight, packed to avoid disturbance during transport and taken to Costa Rica. In San Juan Sur, columns were taken from an experimental treatment (Desnudo) which had been kept free of vegetation for the previous 7 years. The *Centrosema* and Desnudo treatment-plots were chosen for column-samples because they lacked woody roots which would have impeded column extraction and influenced water transport during column leaching.

Prunings:

Prunings were cut from species growing in a Multi-strata agroforestry system established about 250 metres from the improved fallow site where soils were extracted at Yurimaguas; the soil and landform classification is common to both sites. Pruning species were the same as those employed in Chapter 2: *Centrosema macrocarpum*, *Inga edulis*, and *Colubrinaglandulosa*; physical description and use of these species is described in Chapter 2. Prunings were air dried in the shade and transported to Costa Rica. Prior to their application into columns, prunings were cut by hand to pass through a 5 cm diameter mesh.

Design, Set-up and Incubation:

A glass fibre filter paper (Whatmann GF/C) was placed in contact with the soil at the bottom of each column, and held in place by 0.5 mm nylon mesh fastened to the column bottom with a hose clamp. The nylon mesh had been washed by weak HCl followed by distilled water. Washed (weak HCl followed by distilled water) fine sand was poured down column sides where possible to reduce preferential water flow between the soil and PVC side-wall. Sand was inserted until it completely occupied all free space; the masses of sand inserted were small relative to the mass of soil core (0.5 to 4.5 % for Ultisols and 0.7 to 5.1 % for Andisols). Assembled columns were placed in individual containers in 3 cm

water and allowed to wet overnight. During the following day, the water level outside the column was incrementally (3 cm/hour) increased until the column soil depth was reached and the columns completely saturated. Columns were maintained at saturation in containers for 5 hours before being removed to allow drainage of free water; columns were equilibrated for 7 days at field capacity in an open shaded shed before the beginning of the incubation.

The experiment was arranged in a factorial design with 4 completely randomized blocks. Each block contained 10 experimental units: 2 soil types (Ultisol and Andisol) and 5 pruning treatments (Control (no prunings), *Colubrina*, *Inga*, *Centrosema*, and Mix (equal dry mass proportions of *Inga*/*Centrosema*)). Columns were installed on top of funnels allowing filtration of leachate at the column-funnel interface, and easy collection of leachate below the funnel; column tops were continuously covered with plastic plates and leachate was drained into new plastic bags sealed to the funnel spout with tape (See Appendix 5, photo # 11). The air-dry moisture content of each species' prunings was determined and prunings applied to columns at a rate of 1.2 kg/m^2 (oven dry basis) on February 25 1998. Prunings were placed on the soil surface and not mixed with the mineral soil. Immediately following their placement into columns, prunings were wetted with 5 ml water by mist sprayer; this was considered time 0 for the incubation. During the incubation, columns received about 3 ml water by hand sprayer each morning except for weekends. Maximum and minimum air temperature near the columns was recorded daily and ranged between 21° C (standard deviation 2° C) and 33° C (standard deviation 5° C). Distilled water was eluted through the columns 9 times over a 12 week incubation: columns were leached weekly for the initial 6 weeks, and bi-weekly for the last 6 weeks. The first sample date was 03 March 1998. At each leaching water was applied in hourly 140 ml increments until a quantity equal to the calculated soil pore space had been added (Table 4.3). Leachate samples were collected in the morning following leaching (allowing 14-16 hours drainage time), and split for analysis of mineral N and total N.

Table 4.3 Pore space calculation and rate of water application (mm/hour).

Property	Andisol	Ultisol
Column Area	91.6 cm^2	81.1 cm^2
Column Volume	1374 cm^3	1217 cm^3
Soil D_b	0.63 g/cm^3	1.40 g/cm^3
Soil D_p	2.31 g/cm^3	2.65 g/cm^3
Porosity	72.73 %	47.17 %
Pore space	999 cm^3	574 cm^3
Volume added	999 ml	574 ml
Rate of addition (mm/hour)	15.3 mm/hour	17.3 mm/hour

Laboratory Analysis, Data Modeling, and Statistical Analysis:

Analysis of nitrate and ammonium (mineral N) in leachate was completed by distillation using magnesium oxide and Devarda's alloy followed by titration for ammonium (Keeney and Nelson 1982). Analysis for total N in leachate was attempted using alkaline persulfate oxidation (Koroleff 1983; Cabrera and Beare 1993).

Following the incubation, 3 blocks of columns were disassembled, and the 0-2 and 2-7 cm layers separated, homogenized, and analyzed for KCl extractable ammonium and nitrate , and water soluble organic N. Ammonium and nitrate were extracted by shaking 10:1 1M KCl/soil (volume/mass) for one hour followed by ammonium and nitrate determination by distillation/titration. Water soluble organic N was extracted by shaking 5:1 distilled water/soil for one hour followed by Kjeldahl digestion, distillation and titration of the extract for ammonium and nitrate.

The 4th block received cationic Methylene blue dye solution to mark patterns of water flow within columns. Methylene blue readily sorbs to soil surfaces and stains soils a bright blue colour. 6 pore volume-equivalents of 1.21 g/l Methylene blue solution were applied to each column: Ultisols received 3.03 g Methylene blue per column and Andisols

received 6.41 g Methylene blue per column. Columns were allowed to drain for 2 days before they were disassembled and cut into cross section (2 and 7 cm depths), and along their long axis, for inspection and photography of dye deposition.

Exponential and a modified Gompertz model were fit to the leachate data for the period of the incubation. The two models were compared by testing the significance of the F ratio of the difference between the models' residual sum of squares divided by the residual mean square of the more complex model (Burton and McGill 1989). Exponential models assume that maximum mineralization rate is always at time 0. In contrast, the modified Gompertz model allows maximum mineralization rates to occur after time 0. The modified Gompertz model allows calculation of a number of variables including time to maximum mineralization rate, and time lag (time to maximum mineralization minus time to maximum mineralization if mineralization rate increase were greatest at time 0). A graphic illustration of cumulative and incremental data, time to maximum mineralization rate, and lag time for Ultisol-*Colubrina* data is presented in Appendix 8.

Equation 4.1 Modified Gompertz model for incremental N release (Ellert and Bettany 1992).

Differential Form: $dN/dt = \mu N e^{-Dt}$

Integrated Form: $I_{it} = N_o e^{k[1 - e^{k(t - i)}]} - N_o e^{k(1 - e^{kt})}$

Equation 4.2 Exponential model for incremental N release

Differential Form: $dN/dt = kS$

Integrated Form: $I_{it} = N_o(e^{(-k*t)})(e^{(k*i)}-1)$

Equation 4.3 Time to maximum mineralization rate, Gompertz model (days)

$T_{mm} = 1/k \ln(k/\alpha_o)$

Equation 4.4 Lag time, Gompertz model (days)

$$T_{lag} = 1/k[\ln(k/\alpha_o) - (1/(e(\alpha_o/k-1))) +1]$$

Equation 4.5 Maximum Rate of N Mineralization (mass/day)

$$Rm = N_o k[e^{(\alpha_o/k-1)}]$$

Where (for equations 4.1 to 4.4):

α_o = specific rate of mineralization at time 0 (kg/ha/day)
D = growth or substrate reduction constant ($time^{-1}$)
e = base of natural logarithms (e^1=2.718281828)
h = proportionality constant (α_o/k) (Gompertz only)
i = time period between leachings (days)
I_{it} = incremental quantity of N in column leachate during the interval *i* preceding time *t* (kg/ha)
k = (Gompertz) acclimatization coefficient defining the increase in α through time ($days^{-1}$); (Exponential) specific mineralization rate ($days^{-1}$).
ln = natural logarithm
N = mass of nitrogen (mass)
N_o = potentially mineralizable organic N at time 0 (kg/ha))
R_m = maximum mineralization rate
S = substrate (mass)
t = cumulative incubation time (days)
T_{mm} = time to maximum mineralization rate, Gompertz model (days)
T_{lag} = time to maximum mineralization rate minus time to maximum mineralization rate if rate of mineralization rate increase is greatest at time 0, Gompertz model (days)

Models were fit to the leachate data using SAS 6.12 software. Interaction between soil and pruning factors was tested using SAS 6.12 software. Regression and correlation between litter characteristics, Gompertz model parameters, and litter characteristics/Gompertz model parameters were completed with Excel 7.0 software.

4.3 Results

Pruning characteristics:

Using the quantities of nitrogen, lignin, and polyphenols determined for each pruning species, the L+PP:N and C:N ratios were calculated (Table 4.4). The *Inga/Centrosema* (Mix) treatment was based on equal proportions of each constituent on a dry matter basis, thus % N, L, and PP results for the mix were calculated as the average of values for *Inga* and *Centrosema*. In contrast to expected characteristics, *Centrosema* prunings contained the least nitrogen and had the highest C:N ratio (21.40). The high quantity of N in *Colubrina* prunings (4.30 %) was not expected as *Colubrina* prunings used previously in Yurimaguas, Peru, contained only about 2/3 of this amount (2.78 %). The *Colubrina* prunings used in this experiment were taken form a single young *Colubrina* tree growing in the understory of a Multi-strata agroforestry system. In contrast, the *Colubrina* prunings employed in the earlier Yurimaguas experiment were cut from a variety of older trees growing in an improved fallow system. Lignin and phenolic constituents tended to be highly positively correlated in these materials (Table 4.5).

Table 4.4 Initial chemical characteristics of prunings applied to soil columns. Results for each constituent are % of total tissue dry mass.

	%N	% Lignin	% Polyphenols	L+PP:N	C:N
Colubrina glandulosa	4.30	16.77	1.83	4.33	11.24
Inga edulis	3.54	36.01	4.04	11.31	13.13
Centrosema macrocarpum	2.71	15.05	0.78	5.84	17.07
Inga / Centrosema Mix	3.13	25.53	2.14	8.85	15.13

Table 4.5 Correlation (r) among chemical characteristics of prunings.

	% Nitrogen (N)	% Lignin (L)	% Polyphenols (PP)
%N	1.00		
%L	0.13 n.s.	1.00	
%PP	0.34 n.s.	0.98 *	1.00

* - Significant at $P \leq 0.05$

n.s. - Not significant at $P \leq 0.05$

Mineral N in Control Leachates:

Mineral N (NO_3^- + NH_4^+) in leachate from the Andisol controls declined with time to day 41 and then stabilized (Figure 4.1). In leachate from the Ultisol controls, the amount of N tended to decline to a minimum at day 41, and then increased. Variability in the amount of N in leachates from both soils declined with time. Consistently, nearly half of the mineral N in Ultisol leachate was NH_4^+. In contrast, very little NH_4^+ was found in Andisol leachates (Figure 4.2).

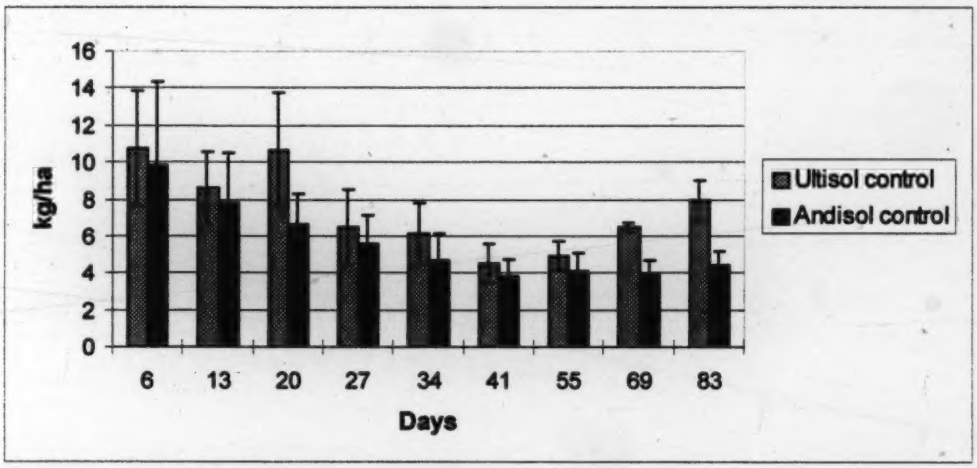

Figure 4.1 Mineral N (NO_3^- and NH_4^+) in leachate collected from Andisol and Ultisol control soils (no prunings added).

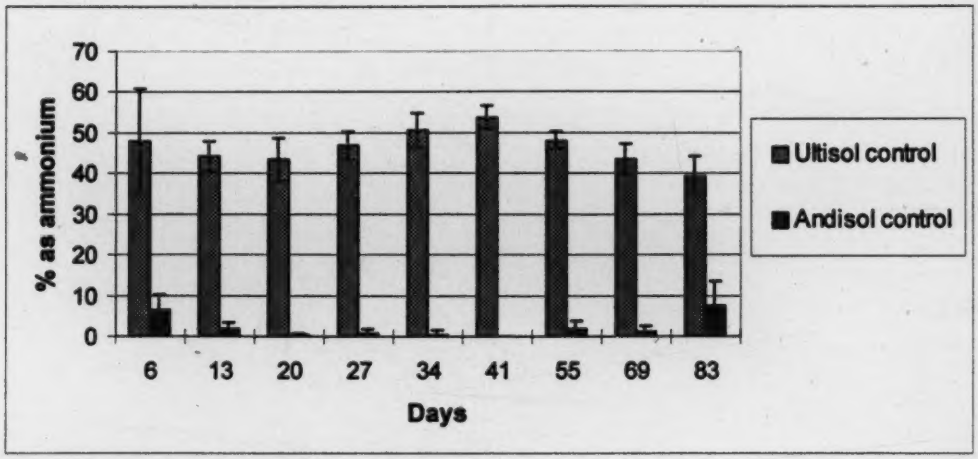

Figure 4.2 Percent of leachate mineral N as NH_4^+.

Mineral N in Treatment Leachates:

Mineral N in leachate from control soils was subtracted from values for the respective treated soils yielding N in leachate attributable to release from the pruning treatments. Values were normalized for the total amount of N added in each pruning treatment and expressed as % of added N (Figures 4.3 and 4.4).

Patterns of mineral N in leachate from Ultisol columns differ from patterns of mineral N in Andisol leachate. Rates of mineral N release from *Inga*, *Centrosema* and Mix treatments on Ultisol columns converge on each other as they near the last weeks of the incubation (Figure 4.3). In contrast, rates of mineral N release from *Inga*, *Centrosema* and *Colubrina* treatments on Andisol columns parallel each other throughout the incubation (Figure 4.4). Andisol columns always released less mineral N than Ultisol columns, with the exception of the Mix treatment, where more mineral N was measured in Andisol leachate (Figure 4.7). Soil-pruning (Mix) interaction was statistically significant ($P \leq 0.05$) in the incremental data at days 13 and 20, and in the cumulative data to day 83 (the end of the incubation). In both soil types, the proportion of mineral N as NH_4^+ declined gradually with time (Figures 4.5 and 4.6). NH_4^+ proportions from Andisols were always lower than Ultisols.

With the exception of the Ultisol-*Centrosema* treatment, the rates of mineral N release to leachate of all soil-pruning treatments increase from Day 6 to Days 55 and 69. Thus maximum mineralization rates did not typically occur at time 0 as an exponential mineralization model assumes. Rates of N release to leachate of all soil-pruning treatments decline after Day 69. The *Colubrina* pruning treatment released N to the leachate of both soils in a very similar pattern. *Colubrina* release rates increase rapidly during the mid-point of the incubation and rates drop sharply after day 69. A fungal infestation became visible in *Colubrina* treatments which corresponded with this period of rapid increase in the rate of N release. At least two generations of a fungus feeding fly, family *Mycetophilidae*, grew in the *Colubrina* treatments.

Collected leachates were often a yellow-green color (See Appendix 5, photo #12). Color was common from *Centrosema, Colubrina* and Mix prunings on Ultisol columns. Andisol leachates were only rarely colored. Leachate color appeared to be related to mineral N concentration, and the color of *Colubrina* leachate corresponded with heavy fungal infection and fly feeding.

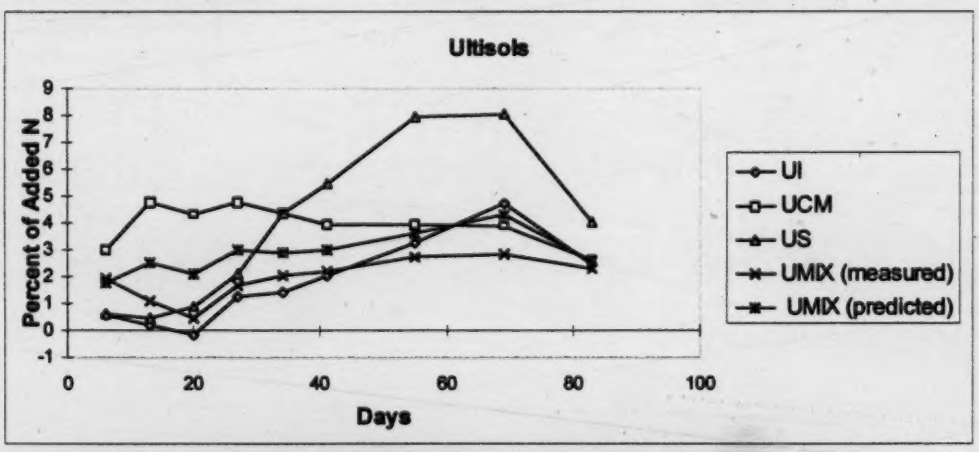

Figure 4.3 Net incremental mineral N (NO_3^- + NH_4^+) in leachate collected from treated Ultisol soil columns, expressed as percent of N added in prunings. UI=Ultisol/*Inga*, UCM=Ultisol/*Centrosema*, UMIX=Ultisol/Mix, US=Ultisol/*Colubrina*. UMIX is the predicted quantity of N in column leachate if no interaction occurs between mix constituents.

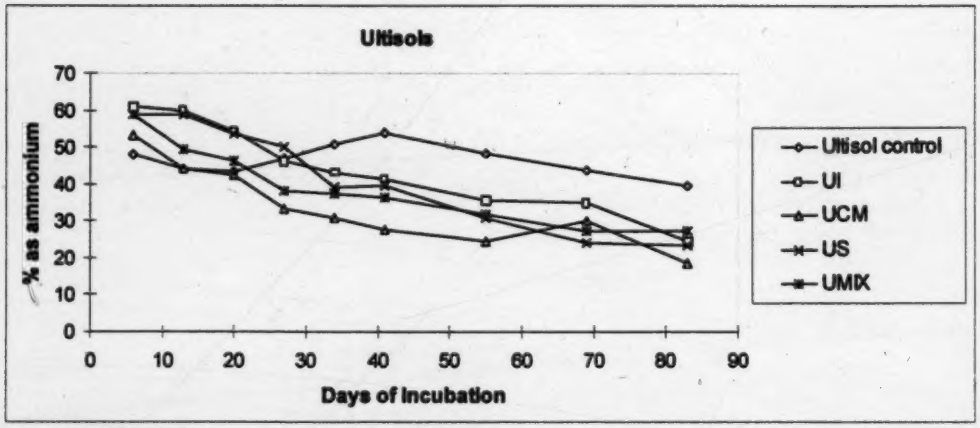

Figure 4.4 Percent of mineral N (NO_3^- + NH_4^+) as NH_4^+ in leachate from treated Ultisol columns.

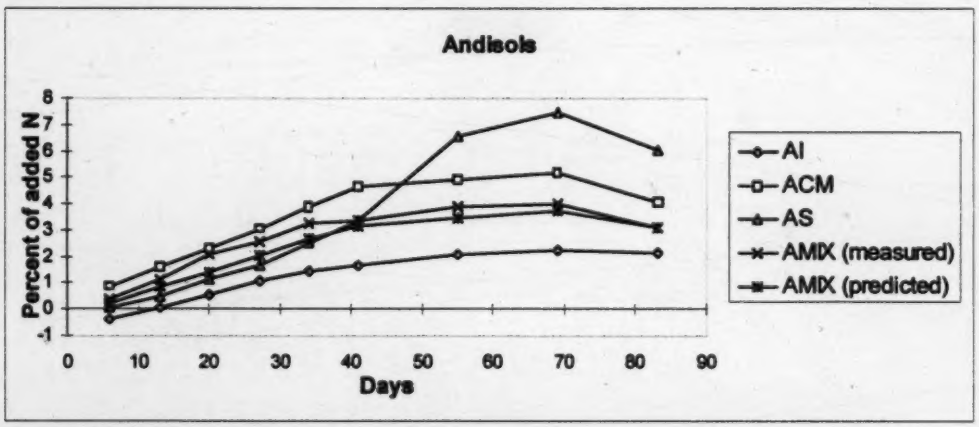

Figure 4.5 Net incremental mineral N (NO_3^- + NH_4^+) in leachate collected from treated Andisol soil columns, expressed as percent of N added in prunings. AI=Andisol/*Inga*, ACM=Andisol/*Centrosema*, AMIX=Andisol/Mix, AS=Andisol/*Colubrina*.

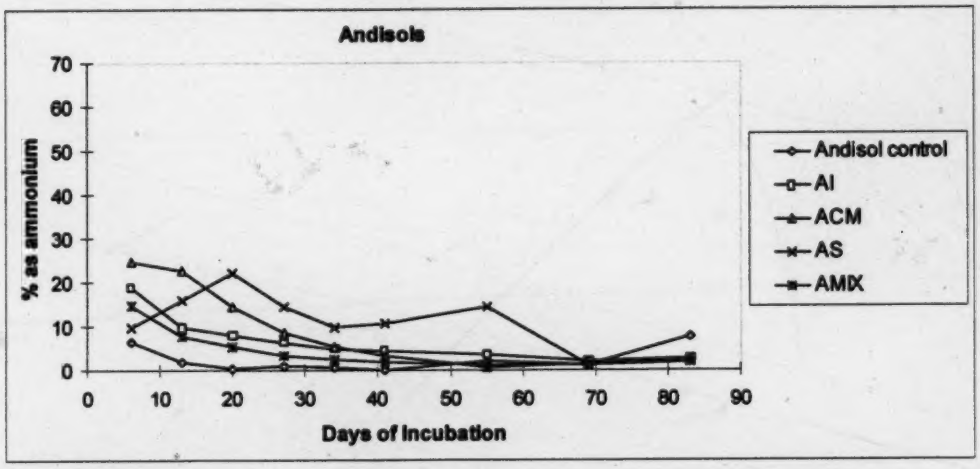

Figure 4.6 Percent of mineral N (NO_3^- + NH_4^+) as NH_4^+ in leachate from treated Ultisol columns.

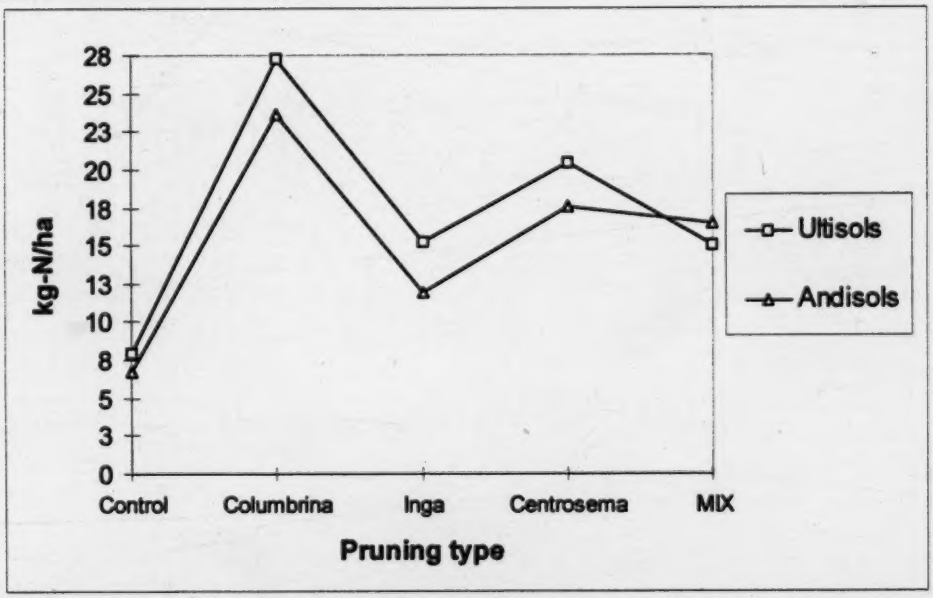

Figure 4.7 Mean quantity (all times) of N in leachate classified by pruning and soil type.

The N release to leachate from the mix treatment can be predicted if there is no interaction between *Inga* and *Centrosema* materials. Predicted and actual amounts of mineral N in leachate from the mix treatments is presented in Figures 4.8 and 4.9. Mineral N measured in leachate from Andisol-Mix treatments was very similar to predicted values. Mineral N in Ultisol-Mix treatments was initially very close to the prediction but the rate of release dropped during weeks 2 and 3 and was consistently smaller than the prediction thereafter.

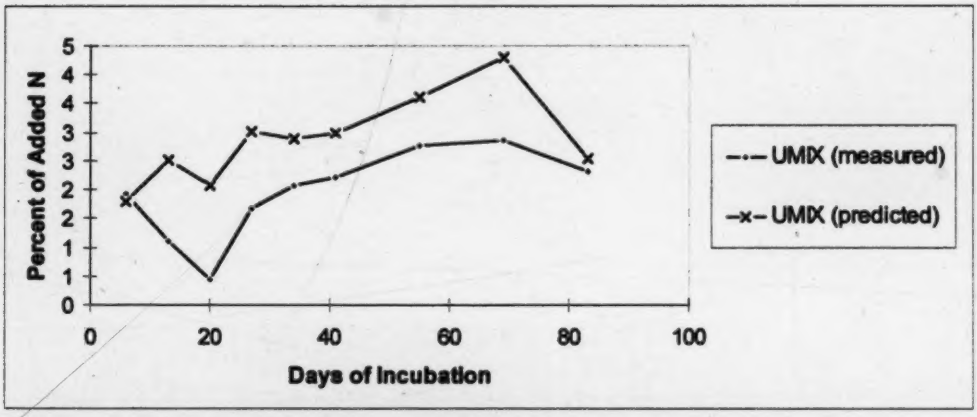

Figure 4.8 Net incremental mineral N (NO3- + NH4+) release from mixed pruning treatments (*Centrosema* and *Inga*) over Ultisol soil columns; the predicted trend is based on individual release rates of components in pure incubations.

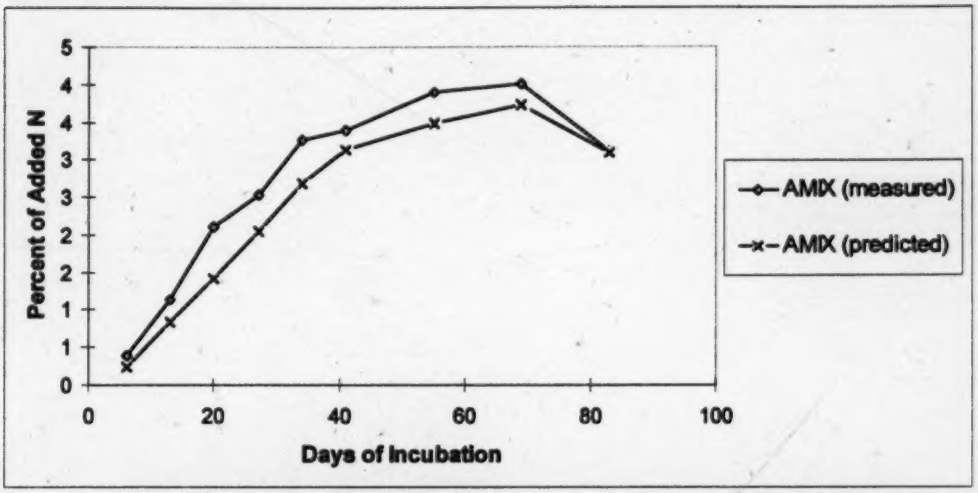

Figure 4.9 Net incremental mineral N (NO3- + NH4+) release from mixed pruning treatments (*Centrosema* and *Inga*) over Andisol soil columns; the predicted trend is based on individual release rates of components in pure incubations.

Analysis for organic N forms in leachates was attempted following the initial 4 sample dates. In almost every sample analyzed, the total N determination by persulfate oxidation yielded less N than analysis for mineral forms by distillation/titration (data not shown). Results from sample analysis and also analysis of urea standards suggest N loss during the procedure. During the persulfate oxidation, N is reportedly transformed very rapidly from ammonium to nitrate with little opportunity for volatile loss (Cabrera and Beare 1993). It is possible that a problem during the colorimetry interfered with nitrate quantification, Cl^- ion interference was encountered during the first attempt at color development in a standard set. It is also possible that oxidation of all N forms to nitrate was not complete, but this problem has not been reported for relatively low N concentrations and the 2:1 sample/oxidizing solution ratios employed (Cabrera and Beare 1993).

Column sectioning:

Soil sampling following the incubation period revealed that concentrations of water soluble organic N were small relative to KCl extractable mineral N (Figures 4.9 and 4.10).

KCl extractable mineral N in Andisols was dominated by nitrate. In Ultisols, appreciable concentrations of both NH_4^+ and NO_3^- were extracted by KCl.

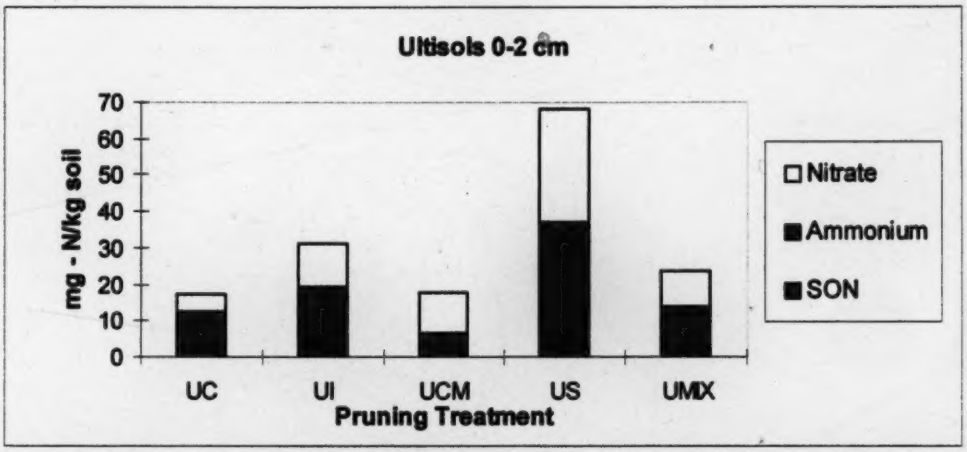

Figure 4.10 KCl extractable NO_3^- and NH_4^+ and water soluble organic N sampled in 0-2 cm layer of Ultisol soil columns after 83 days incubation. UC=Ultisol-Control, UI=Ultisol-*Inga*, UCM=Ultisol-*Centrosema*, US=Ultisol-*Colubrina*, Ultisol-Mix.

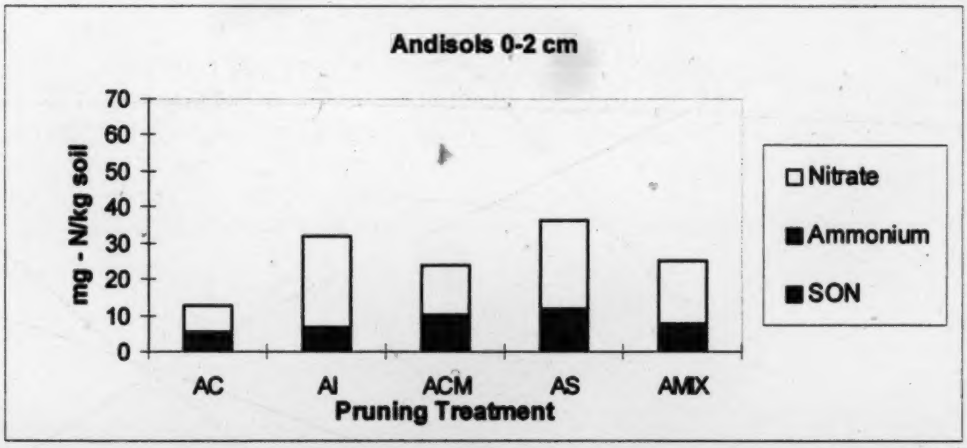

Figure 4.11 KCl extractable NO_3^- and NH_4^+ and water soluble organic N sampled in 0-2 cm layer of Andisol soil columns after 83 days incubation. AC=Andisol-Control, AI=Andisol-*Inga*, ACM=Andisol-*Centrosema*, AS=Andisol-*Colubrina*, AMIX=Andisol-Mix.

Mass loss of the pruning materials was ranked *Colubrina*>*Centrosema*>Mix>*Inga* (Table 4.6). No recognizable *Colubrina* tissue remained at the end of the incubation period and thus mass loss was considered to be 100% (see Appendix 5, photo #14). Mass loss of each pruning treatment was significantly different from the others, and the ranking is closely related to the ranking in cumulative total N release to leachate. *Colubrina* lost significantly more mass than *Centrosema*, but cumulative N release (normalized for initial quantities of N added in prunings) from *Centrosema* was equal to or slightly larger than *Colubrina*.

Table 4.6 Percent of initial mass lost during incubation*.

	Colubrina	*Inga*	*Centrosema*	Mix	Mix (predicted)
Ultisols	100 (0)	21.3 (0.3)	68.0 (12.4)	42.6 (2.4)	44.6 (6.4)
Andisols	100 (0)	25.9 (2.1)	69.2 (6.0)	48.0 (1.2)	47.6 (4..0)

*Mass remaining was defined and measured as recognizable plant tissue on soil surface after 83 days. Brackets indicate the standard deviation of the measurement.

Dying thoroughly stained the top 1 to 2 cm of both Andisol and Ultisol columns. In both soil types staining was more marked along 0.5 - 1.0 cm of the column side than internally. While breakthrough was achieved in the Ultisol columns after 2-3 pore volumes, dye did not break through in the collected leachate of some of the Andisol columns. Where dye did break through from Andisol columns it occurred in the last 2 pore volumes. Andisols were more strongly aggregated than Ultisols and were stained predominantly along cracks between aggregates and to a lesser degree internal to aggregates. In contrast to the Andisols, Ultisol columns contained large *ca* 0.5 cm channels from macro-faunal activity and many tiny root hairs. A portion of the root hair-channels and macro-fauna channels in Ultisols were completely stained while other channels were not contacted by the dye (See Appendix 5, photo # 13). Preferential flow along channels was a marked feature of the stained Ultisols. See photos in Appendix 5.

Modeling:

A modified version of the Gompertz model developed by Ellert and Bettany (1992) for incremental N release fit all data sets, except for the Ultisol- *Centrosema* treatment and the Mix treatments, significantly better than did the exponential model (see Equations 3.1 and 3.2, Table 4.7). The Gompertz model yielded values for N_o (normalized to g mineral N per kg N added) that were close to the measured values. In contrast, the exponential model yielded N_o values that were too high to be realistic. *Inga* and *Colubrina* pruning treatments exhibited the longest lag times on both soil types (Table 4.8). *Centrosema* and *Colubrina* had the highest mineralization rates (in percent of added N).

In Andisol columns, C:N ratio was significantly correlated with lag time and inflection time, and L+PP:N ratio was significantly correlated with maximum mineralization rate (Table 4.9) None of the other correlations between maximum mineralization rate, lag and inflection time, and chemical indices were significant. Lignin + Polyphenol:N ratio was strongly negatively correlated with N_o (Table 4.10).

Table 4.7 F and r2 statistics for modified Gompertz and exponential models for incremental N release. A significant F ratio means the modified Gompertz model better describes N release than the Exponential model. No units are g/kg of N added.

Treatment	F ratio †	r^2†† (Gomp.)	r^2 †† (Expon.)	Measured Cumulative N release	No (Gomp.)	No (Expon.)
U-S	24.95 **	0.84	0.72	340	346	8959
U-I	16.45 **	0.77	0.66	158	159	466
U-CM	2.41 ns	0.84	0.83	357	365	17584
U-Mix	0.54 ns	0.72	0.71	174	206	789
U-Mix (predicted)	4.41 *	0.89	0.88	244	267	662
A-S	22.78 **	0.85	0.75	293	322	8087
A-I	15.79 **	0.85	0.78	110	124	4951
A-CM	10.95 **	0.91	0.88	305	324	21586
A-Mix	-2.00 ns	0.90	0.91	239	252	9023
A-Mix (predicted)	31.82 **	0.90	0.81	194	210	3465

† F-ratio calculated as the difference between the residual sum of squares of the modified Gompertz and exponential models divided by the residual mean square of the modified Gompertz model (Burton and McGill 1989).

†† r^2 was calculated as the sum of squares due to regression divided by the total sum of squares.

** Significant at $P \leq 0.01$

n.s. Not significant at $P \leq 0.05$

Table 4.8 Lag time, inflection time, and maximum N release per day in both kg/ha and % of added N. See Appendix 8 for an illustration of these variables.

	modified Gompertz model				
Treatment	Inflection Time * (days)	Lag Time (days)	Max. Rate (kg/ha/day)	N application (kg/ha)	Max. Rate (%/day^{-1})
U-I	59	29	1.38	425	0.33
U-CM	-10	0	2.19	325	0.67
U-S	53	21	3.37	516	0.65
U-MIX	37	2	0.85	375	0.23
U-MIX (predicted)	33	2	1.26	375	0.34
A-I	59	18	0.76	425	0.18
A-CM	45	7	1.41	325	0.44
A-S	61	26	2.87	516	0.56
A-MIX	44	7	1.30	375	0.35
A-MIX (predicted)	50	11	1.07	375	0.29

* Time of maximum rate of N mineralization.

Table 4.9 Correlation (r) between chemical characteristics and model estimates of inflection time, lag time, and maximum rate of N release.

	Inflection Time		Lag Time		Max. Rate	
Indice:	Ultisols	Andisols	Ultisols	Andisols	Ultisols	Andisols
% L + PP	0.64 ns	0.27 ns	0.52 ns	0.08 ns	-0.63 ns	-0.67 ns
L+PP:N	0.37 ns	-0.07 ns	0.20 ns	-0.27 ns	-0.85 ns	-0.87 *
C:N	-0.86 ns	-0.92 *	-0.82 ns	-0.95 **	-0.50 ns	-0.57 ns

** Significant at $P \leq 0.01$

* Significant at $P \leq 0.05$

n.s. Not significant at $P \leq 0.05$

Table 4.10 Correlation (r) between modified Gompertz model parameters and pruning characteristics.

	k	h	No
L+PP:N	-0.02 n.s.	-0.16 n.s.	-0.92 **
C:N	-0.81 **	0.71 *	-0.42 n.s.

** Significant at $P \leq 0.01$

* Significant at $P \leq 0.05$

n.s. Not significant at $P \leq 0.05$

4.4 Discussion

Possibly the most serious problem with using the first order exponential form for N mineralization data is that it does not allow for a lag in mineralization rates (Ellert and Bettany 1988). Visual inspection of published N release curves for agroforestry materials reveals that lags are common in materials with C:N ratios low enough to allow immediate net mineralization. High concentrations of lignin and polyphenols are believed to reduce mineralization rates, but very little attention is paid to the shape of N release with time when control by lignin or polyphenols is apparent. Fitting equations to mineralization results not only allows concise description of data, but also the opportunity to further describe biophysical phenomena if models are connected with characteristics of the ecosystem under investigation.

The shape of mineralization curves can be expected to be controlled by substrate availability to microbes, and thus fit a first order exponential function, if materials are homogenous, progressively decrease in quality, and communities of decomposers are large. However foliage is not homogenous but highly structured, with lignin and cellulose in cell walls surrounding more labile materials inside. It may be hypothesized that materials with much cell wall lignin exhibit sigmoidal N release curves during decay, in relationship to the order in which decomposers gain access to different types of substrate. Alternatively, the ability to degrade lignin and polyphenols varies on a decomposer-specific basis. Thus lag time could be related to adaptation of the microbial community to a particular substrate. Much investigation of lignin and phenolic control of mineralization rates has relied on oven dried and finely ground materials. Drying may alter mineralization rates (Taylor 1998), and ground materials are rendered much more structurally homogenous (Fyles and McGill 1986) than they are as prunings or litter in agroforestry systems, possibly yielding artificial N release curves which fit the exponential model.

Lignin + Polyphenol:N ratio was significantly correlated with modeled N_o ($r=0.92$) while C:N ratio was not. This is in agreement with the findings of field experiments conducted

with the same materials (Chapter 2) and recently published work on N release from agroforestry litters and prunings (Fox et al. 1990; Constantinides and Fownes 1994; Handayanto et al. 1994 and 1997; Lehmann et al. 1995). Some authors suggest that mineralization from low C:N legume foliage is controlled primarily by the action of polyphenols (Palm and Sanchez 1990 and 1991); lignin and phenolic contents were highly correlated in the materials used here so differentiation between them was not possible. C:N ratio was not a good predictor of cumulative N release, but was highly negatively correlated with inflection time and lag time of the modified Gompertz model fitted to the data. C:N ratio was also correlated with k ($r=-0.81$) and h ($r=0.71$) which are related to the shape (lag time) of the Gompertz mineralization curve. N release from low N content materials (*Centrosema*) was stable compared to N release from *Colubrina*, from which N release rates were very dynamic during the incubation.

Northup et al. (1995) found that the ratio of DON to mineral N in incubation extracts varied positively with litter phenolic concentration. It is possible that decomposing *Inga* prunings release large proportions of DON relative to mineral N, because of their high polyphenol content. Because leachates were collected below 15 cm soil columns, it was not expected that large amounts of DON would be measured in leachates. DON measurements were attempted in this experiment (data not shown) but mineral N analysis (distillation/titration) yielded more N than total N analysis by persulfate oxidation, indicating that either DON was not present or analysis was not reliably achieved. Some leachates were darkly colored during the incubation suggesting DOC release, and DON is a subset of DOC. However, darkly colored leachates were not common from high phenolic (*Inga*) treatments. Because DON readily sorbs to soils (Fahey 1985; Northup et al. 1995) it should have been recoverable in analysis of column soils if it was not mineralized. Soil analysis for KCl extractable mineral N and soluble organic N suggest only low concentrations of soluble organic N (or DON) relative to mineral N, and no increases under *Inga* or the Mix treatment relative to the others. Some other workers have not found increased N concentrations below vegetation high in tannins (Caroline Preston -

personal communication). Not all polyphenols readily bind proteins (Handayanto et al. 1994; Reed 1995) and the strength of protein-phenol complexes depends on properties of both the protein and the phenolic compound (Zucker 1983; Reed 1995). In summary, these data do not support the hypothesis that DON production and movement is significant. This must be tempered by the fact that recovery of DON in the analysis used may have been incomplete.

The stability of protein-polyphenol complexes in the soil is likely highly dependent on specific conditions. Howard and Howard (1993) found that the degradability of polyphenol-gelatin complexes was highly dependent on the species source of the polyphenols; complexes formed with pruning extracts of some species were readily degraded by the non-specialized microbial community of distilled water while other species extracts formed complexes highly resistant to ammonification. Leake and Read (1989) found that an ericoid fungus *Hymenoscyphus ericae* was readily able to utilize protein from various types of phenolic-protein mixtures. *Hymenoscyphus ericae* was able to degrade tannic acid while an ectomycorrhizal fungus exhibited only limited degradation of tannic acid (Bending and Read 1996). Saprophytic fungi were able to degrade protein-polyphenol complexes, ericoid fungi were capable of partial degradation of the complexes, and only a few of the tested ectomycorrhizal fungi could degrade protein-polyphenol complexes (Bending and Read 1996). In this experiment, it is possible that protein-polyphenol complexes were formed and subsequently degraded either upon contact with the soil surface or in the mulch itself. Phenolic binding reactions could have caused an accumulation of DON which was only temporary (for example 1 to 3 weeks) and thus not evident in soil sampling completed after 12 weeks. The pattern of mineral N release to leachate was prominently sigmoidal for Ultisol-*Inga* and Ultisol-Mix treatments.

If there is no interaction between components, mixed pruning treatments will release the mean of their components' individual release patterns. Interaction between mix components is apparent when nutrient release is significantly higher or lower than the

mean-prediction; both higher and lower than non-interaction predictions are hypothesized to occur. In this experiment there did not appear to be interaction between mix components in Mix-Andisol columns, as measured amounts of N in leachate were very close to those predicted by non-interaction. Results from Mix-Ultisol columns were unpredictable under the hypothesis of non-interaction, and the low levels of N release to leachate are consistent with phenolic action. The exponential model fit Mix-Ultisol results equally as well as the modified Gompertz model, but the predicted Mix-Ultisol results fit the Gompertz model significantly better. Rates of N release from the Mix-Ultisol drop in the first 3 weeks of incubation, and are lower than non interaction predictions throughout (except for day 6). Mass loss from the Mix-Ultisol was slightly greater (not statistically significant at $P<0.05$) than predicted mass loss but cumulative specific N release to leachate was 70 g/kg lower (not significant at $P<0.05$) than the non-interaction prediction. Further, the Mix-Ultisol treatment was the only pruning treatment in Ultisol columns to release less mineral N to leachate than its counterpart treatment in Andisol columns.

It is difficult to speculate why interaction would occur in Ultisols but not Andisols. There are marked differences between classes of fungi in their ability to degrade phenolic complexes (Bending and Read 1996); the Andisol may have contained a different class of fungi than the Ultisol. The prunings applied to both soils were of the same origin, so there were at least some common microbial innoculum. But even if microbial communities were very different, protein-phenol complexes would have caused a short lag (lag in excess of non-interaction predicted lag) in mineral N release to leachates. No such lag is visible in the Andisol results, suggesting that either complexes did not form or were degraded immediately on contact with the soil surface.

Both soils have very low base status, high aluminum saturation and low pH (Andisol pH = 4.2, Ultisol pH=5.2). A distinct characteristic of the Andisol is the influence of volcanic ash and the presence of allophane minerals which are believed to sorb and physically protect soil organic matter from decomposition. But increased sorption and physical

protection by the Andisols is not consistent with the lack of an interaction-lag in Mix-Andisol treatments. Reed (1995) notes that protein-tannin complexes may be stable at the pH of the rumen, but not at lower pH in the abomasum. Northup et al. (1995) found that DON is composed of protein-tannin complexes which were intact at ambient soil pH (4.5) but dissociated at lower pH values. The pH of the Andisol was recorded at an order of magnitude lower than the Ultisol, but 4.2 may not be sufficiently low to cause dissociation of protein-tannin complexes.

4.5 Conclusions:

L+PP:N was better correlated with mineral N release, but C:N ratio was negatively related to the length of time from the beginning of decomposition to the maximum rate of N release. Therefore both of these indices may be critical considerations in managing N release for synchrony. These results do not support the hypothesis that DON is a large proportion of the N released from prunings, but analytical problems in determination of dissolved organic N prevent a strong conclusion. Interaction between mixed prunings was possible in Ultisol columns but was not apparent in Andisol columns. If soil type controls interaction between mixed prunings, then the mechanism(s) require further investigation.

Prunings applied to soil columns exhibited lagged mineralization rates. Classical exponential models of N release assume that mineralization is most rapid at time 0 and that mineralization is controlled by substrate availability. Models which account for a mineralization lag are perhaps superior in systems where an array of decomposition controls exist.

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5. Synthesis

5.1 Introduction:

Conserving nutrients within soil-plant systems is linked to the concept of synchronous transformations of nutrients in these systems. Transformations occur during decomposition and plant uptake; either process might be managed for synchrony. Management of decomposition rates for nutrient conservation and synchrony requires that farmers possess a set of management tools with which they can predict and manipulate nutrient release. These tools must be sufficiently robust to have application across diverse ecological gradients. The capacity to fine tune each tool systematically on a site- and region- specific basis will enhance their usefulness. If tools are to gain widespread acceptance, simplicity of use will be an advantage.

Conservation of nutrients within soil-plant systems is a valuable practice because the costs of perturbed nutrient cycles are minimized. In this context, synchrony will be an important concept in future management of forests, agro-ecosystems and agroforestry systems.

Conclusions of this work:

In Chapters 2 and 4, L+PP:N ratio was a useful predictor of N release, but C:N ratio was also related to the timing of maximum N release. Further, controls on decomposition other than the chemical constituents of prunings were apparent. Slash and burn disturbance resulted in a mineral N flush and likely some N loss from the soil plant system. Lysimeters and IEMs produced different measurements of soil N status, and N status was not related to fertility for a maize crop.

Mixing materials of high- and low- polyphenol contents appeared to result in interaction between the components. In chapter 2 interaction resulted in reduced mineralization rates in the first 3 weeks and increased mineralization thereafter. In chapter 4, fitting

mineralization from mixed treatments to models revealed that mixes could not be fit to the same mineralization models as their constituents incubated in pure form. A lag in time to maximum mineralization is a feature of many types of prunings decaying in a mulch layer at the soil surface.

5.2 Managing Decomposition:

Chemical indices:

In order to manage decomposition of plant materials placed on the soil surface, certain chemical characteristics will have to be determined. Among the factors which will compose the farmers "tool pack" for predicting decomposition will be measures of initial chemical constituents of materials such as the quantities of N, C, cellulose, lignin, polyphenols, and protein-binding capacity. These have been widely studied and variously correlated with decomposition and nutrient release (Melillo et al. 1982, Frankenberger and Abdelmagid 1985, Palm and Sanchez 1991, Constantinides and Fownes 1994, Handayanto et al. 1994, Bending et al. 1998).

Among the current problems with chemical indices, however, is the lack of agreement over which are most suitable. A wide range of species may be employed in agroforestry with considerable diversity in chemical composition; the small group of species examined in this thesis grew on the same site and differed two fold in N and four fold in lignin and polyphenol content. Bending et. al (1998) comment that most of the aforementioned chemical constituents have been correlated with N release, depending on the species investigated. In chapter 2 it was found that L+PP:N was correlated with N measured using plate lysimeters and porous-cup suction lysimeters established within an agroforestry system. Thus even in the presence of heterogeneity in microclimate
and soil, chemical characteristics of prunings exerted measurable control on decomposition; 30 - 80 % of the variation in N release was predictable based on L+PP:N ratio.

Since the applicability of a chemical measure depends on conditions specific to the system being studied, perhaps these measures need to be organized in a hierarchy based on the nature in which each constituent controls nutrient release. For example, materials with C:N ratios above 30 are likely to initially immobilize N regardless of their lignin and polyphenol contents. When C:N ratios are low enough to allow net mineralization, then lignin and polyphenolic controls may become significant. Ranges and thresholds at which constituents occur relative to one another also appear to be critical. Both Palm and Sanchez (1991) and Oglesby and Fownes (1992) found that N mineralization was reduced above a critical PP:N ratio of 0.5.

C:N ratio is a fundamental control on N dynamics because decomposition results from the search for energy in C-H bonds. Lignin and polyphenol contents, which can limit C and N availability or cause toxicity (polyphenols), are a secondary control on N release. Thus a hierarchy of indices must integrate the fundamental nature of each control on nutrient release and the ranges at which this control is significant.

To date, most proximate analyses of prunings and litter do not consider the distribution of constituents within plant tissues, or their mobility during decomposition. Soluble materials are likely more available for decomposition or interaction with other substrates (Handayanto et al. 1997), meaning that they are more important at early stages. Protein binding capacity may be among the best indicators of early N release; it combines solubility and function of phenolic constituents, and it is simple to perform, making it a promising candidate among chemical indicator tools for agroforestry system managers.

Heterogeneity in materials implies that the ratios of chemical constituents encountered by decomposers changes with time during decomposition. There is growing evidence to suggest that heterogeneity accounts for considerable variation in decomposition rates is growing (Fyles and McGill 1986; Smith et al. 1998). Heterogeneity may be very difficult

to assess and include in chemical constituent indices. Botanical information may be useful. Morphology of plant structures is species-specific and chemical characteristics are species-specific to some extent. The contrasting lignin and N contents of *Colubrina glandulosa* prunings from old (Chapter 2) and young (Chapter 4) trees emphasizes the need for caution in correlating chemical characteristics to species alone.

The 30-80 % of variation in N release accounted for by L+PP:N in the field experiment reported here falls short of the predictability which may be needed to manage N transfers efficiently. We worked with a small group of materials, which may have improved or hindered correlation. Nevertheless, factors other than litter chemical constituents also controlled rates of N release. Janzen et al. (1994) propose that controls on decomposition can be classified as physicochemical controls, and population- or community-level biological controls. According to this classification, documentation of the controls on decomposition in agroforestry literature is disproportionately skewed to chemical controls. Quantification of other physicochemical controls such as climate and micro-climate are required. As well, population level controls such as differential ability to degrade phenolic constituents may be significant, especially in variable mycorrhizal associations in root systems of tree legumes (Bending and Read 1996). Smith et al. (1998) found that decomposition rates of the same prunings varied depending on the stand type in which they were placed and suggest that microbial communities differed with stand type. Community level controls are also suggested by Janzen et al. (1994). Quantification and integration of physicochemical, population, and community controls on decomposition along with current basic knowledge of chemical controls is required to further explain variability in nutrient release.

Mixing:

Mixing materials of differing chemical composition is a promising tool for management of nutrient release, but there is growing evidence that the behavior of mixes cannot be predicted in the same manner as substrates decomposing in pure form. Work by

Handayanto et al. (1994 and 1997) showed that N release could be manipulated by mixing materials. In this thesis, a 2-4 week lag in N mineralization was sometimes evident, in mixed treatments. No lag was detectable when the mix was used with the Andisol in the column-leaching experiment. Interactions which occur when materials are mixed need further investigation. If interaction occurs as a result of combinations of soluble materials, then the thresholds at which interactions become important need to be known. Handayanto et al. (1997) found no increase in the PBC of a mix until 60% or more was high polyphenolic material. In this work, mixing produced results which could not be well predicted by their L+PP:N content (Chapter 2) and which could not be modeled as well as its constituents (Chapter 4). Interactions resulting from mixing may also result from population or community controls. In Yurimaguas field work mesh size was significantly related to increased N release only from a mix, suggesting that faunal access to the mix was important, in contrast to the same materials placed in pure form. Blair (1990) notes that mixed litters support microbial communities with the ability to degrade a wider range of substrates more rapidly.

Modeling:

Precise use of organic fertilizers in agroforestry systems will require that nutrient release patterns and the controls on release kinetics be quantified. Mathematical models of nutrient release may be a promising management tool. Modeling N release allows quantification of complex release patterns by using a small number of parameters. While indices of chemical control are being quantified, they need to be more closely linked to mathematical models of nutrient release. For example, it is conceivable that future managers will model the kinetics of polyphenolic or lignin control on N release to optimize productivity of agroforestry systems.

Soil Organic Matter build-up:

Investigation of nutrient release from organic fertilizers of agroforestry systems has focused on supplying crop demand in the season of mulch application. Only about 10-30% of the N contained in prunings or litter is transferred to a crop in the same year as they are applied (Palm 1995), consequently these materials may not provide as impressive a crop response as mineral fertilizers. But in contrast to mineral fertilizers from which N not absorbed is potentially lost, the portion of organic fertilizer N not used by crops may be stabilized into soil organic matter. As soil organic matter, this N contributes to the future fertility of a soil in a "residual" or "build-up" effect. Evidence for residual future benefits of organic fertilizers is growing (Haggar et al. 1993; Palm 1995), but these benefits need to be quantified. Quantification of SOM build-up benefits will be a further tool with which managers of agroforestry systems will be able to optimize use of prunings and litter.

5.3 Managing Crop Demand

Matching crops to ecosystems:

Synchrony which allows nutrients to be conserved within the soil plant system depends on both the nature of nutrient storage in the soil and the site vegetation which will eventually recycle soil nutrients. Planning for synchronous nutrient transfers must include a process of matching crop uptake capabilities with basic features of nutrient storage and mobilization which are specific to ecosystems. In comparison to management of decomposition rates, there are at least equal opportunities to manage crop nutrient demand. For example crops can be selected for rhizosphere associations, rooting habit, and life cycle, on a species- or variety-specific basis. Sanchez and Salinas (1981) list among their principals of low-input technology, selection of crop species and varieties tolerant to acid soils and suited to humid moisture regimes.

Slash and burn disturbance studied in this work caused a periodic flush of mineral N in the soil profile, some of which was lost from the soil-plant system. Perennial crops may have a number of advantages over annual cropping systems in acid, infertile, perudic conditions. First, disturbance and resulting nutrient loss during establishment of perennial crops would be less frequent and possibly less severe. Second, a perennial root system may better avoid leaching losses, similar to the structure of unmanaged humid forests. Third, mycorrhizal associations may have more opportunity to establish in perennial systems. Mycorrhizal associations may help the crop exploit a larger volume of soil, and nutrients held in protein-phenol complexes (Northup et al. 1995). Limited leaching and accumulation of N, P, K, Ca are reported in high yielding Brazilian cacao plantations (Santana and Cabala-Rosand 1984). Perennial root systems have been observed to respond rapidly to nutrient pulses (Lodge et al. 1990).

If low input technologies are to succeed in the Humid Tropics of the Amazon basin, then crops which simulate the structure and functioning of unmanaged humid forests may be the most suitable. Humid forests are excellent examples of synchronous nutrient cycles, as nutrient losses are typically small and essentially nutrient cycling is "closed" within the system (Myers et al. 1994). Crop species which have perennial nutrient uptake, deep rooting and beneficial rhizosphere or mycorrhizal associations may be better suited to most soils of the Amazon basin than cereal crops which are more suited either to flood plains (rice) or to semi-arid or sub-humid climates (maize).

Crop Development:

Amid the plant diversity of the Amazon region it seems that many valuable and underutilized potential crops must exist. As access into the region improves, landless in-migrants arrive in search of land - at this subsistence level basic food crops have received the most attention. For the region as a whole, forest products such as tree and shrub fruits, bark and wood extracts, and wood fibre may be more profitable than annual cereal crops. The competitive advantage of humid tropical wood production is increasingly

recognized. Peach Palm (*Bactris gasipaes*) is a prominent example of a local species which grows well even on acid infertile soils and is potentially profitable. The development of commercial crops, however, requires infrastructure to support local producers in product development, marketing, and transportation. Transportation on a very poor road is one of the greatest production costs for Yurimaguas producers (L. Arevalo - personal communication).

5.4 Economics, energy, and nutrient cycles

Lack of farmer adoption is a current criticism of agroforestry technologies (Sanchez 1995). Farmers' process of adoption decisions is complex and includes factors such as profitability, risk, tenure security, capital requirement and availability, and net present value. Farmers must be considered to be rational decision makers responding to the social and market values of their community. Conventional means of agriculture and forestry production provide the majority of the worlds food and fibre, and new or old agroforestry technologies will be judged against this norm. Thus agroforesty systems must have more value to producers than conventional systems before they will be readily adopted.

Manipulation of energy, water and nutrient supplies in agrarian production perturb their flow and cycles in larger ecosystems (McGill and Myers 1987). Current production systems which have allowed world population to more than quadruple in this century rely extensively on manipulated supplies of energy, water and nutrients. Some current environmental problems, or social costs, are associated with disturbed element cycles. CO_2 concentrations in the earth's atmosphere are increasing and this believed to be influencing global climate. Use of mineral N fertilizer can pollute water supplies creating human health risk and damaged marine ecosystems (Pfeffer 1992). Fixation of mineral N fertilizers from the air requires massive energy expenditure, potentially increasing CO_2 emission to the atmosphere.

One of the reasons that agroforesty systems appeal to academics is the principle of sustainable production available to the poorest classes. But before agroforestry alternatives are attractive to producers they must be able realize the value in any reduced environmental costs. In economic terminology, an externality is a side effect of production on parties other than the producer or consumer of the good produced (Blomqvist et al. 1987). External side effects are not accounted for in normal market behavior, and social costs and benefits may differ from private benefits and costs (Mercer and Hyde 1991). CO_2 emissions, water pollution, and soil erosion are costs which are currently external to most producers markets, but constitute a major social threat. When farmers are faced with the choice between agroforestry and conventional systems; externalities probably still allow conventional production alternatives to be relatively more attractive than agroforestry.

Even optimistic projections suggest that in the coming century the world hunger problem will include an overall production shortages as well as the current distribution problems and regional food shortages (Kendall and Pimental 1994). As well as hunger and malnutrition, many national economies are depressed after recent rapid globalization of capital flows; under- and unemployment are common characteristics of the developing world.

The solutions to food shortage and unemployment will include intensification of the production inputs, including labour, per unit land area. Agroforestry systems can maintain and improve soil fertility and may be the most sustainable platform for intensification. They have the potential to support labour investment across a range of land capability classes. Biochemical technical change is believed to be the most appropriate means of improving agricultural productivity in developing countries (Hayami and Ruttan 1988).

Ultimately, agroforestry production will be superior to alternatives in many situations because it can be more harmonious with terrestrial energy flows and nutrient cycles.

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6. Appendix 1: Analysis of covariance technique

Description of analysis of covariance technique used to analyze Chapter 1 lysimeter data:

The analysis of covariance allows comparison of different regression lines, where each experimental treatment can be fitted to a different regression line. Essentially, the analysis of covariance combines the techniques of analysis of variance and regression. Once treatments are fitted to regression lines, the distance between lines (or the difference between them) can be tested at specific points along the X axis. In these data, mineral N concentration in lysimeter samples (Y axis) is regressed on time (X axis). In order to determine the type of comparisons which can be made (the form of the covariance model) several hypothesis must be tested, in order:

1) Test the hypothesis that the regression slopes are not significantly different from 0 (ie. the regression is not significant). If this is not rejected, then treatments can be compared by analysis of variance without considering X (in this case, time).
2) Providing 1) is rejected, test the hypothesis that slopes are parallel (or equal to each other). If this is not rejected, then treatments can be compared at their Y intercept (at time 0) or by their adjusted means.
3) Providing 1) and 2) are rejected, the treatments can be compared at different values of X (at different times).

The SAS statements required for these steps are as follows:

(variables: TRT = mineral N concentration by treatment, DAY= days of incubation)

- Test for slopes = 0

```
PROC GLM
CLASS TRT
MODEL PERCN = TRT DAY TRT*DAY
```

- Test for parallel slopes

```
PROC GLM
CLASS TRT
MODEL PERCN = TRT  DAY  DAY*TRT;
```

- Comparison of TRTs at a given day

```
PROC GLM
CLASS TRT
MODEL PERCN = TRT  DAY TRT*DAY
ESTIMATE  '1-2 at day 13'  TRT  1 -1  0  0  0 DAY*TRT 13  -13  0  0  0;
ESTIMATE  '1-3 at day 13'  TRT  1  0 -1  0  0 DAY*TRT 13    0 -13 0 0;
...etc.
```

Note that the test for 0 slope indicates that all treatment regressions except for the Mix were significant and negative (Table). In the Mix treatment the slope of the regression was not different from zero, thus no significant variation in mineral N concentration with time. Note the r^2 values for the strength of each treatment regression which were low in general.

Table 6.1 Estimates of slope and intercept, and r^2 values for analysis of covariance regressions.

Parameter	Treatment	Estimate	T for Ho (slope= 0)	Pr > |T|	r^2
Intercept	*Colubrina*	0.374	1.53	0.1297	
	Inga	1.035	4.25	0.0001	
	Centrosema	1.475	6.05	0.0001	
	Mix	0.120	0.49	0.6234	
	Pred. Mix	1.303	5.34	0.0001	
Slope	*Colubrina*	-0.020	-2.81	0.0065	.54
	Inga	-0.023	-3.28	0.0017	.28
	Centrosema	-0.018	-2.55	0.0133	.32
	Mix	0.011	1.58	0.1191	.18
	Pred. Mix	-0.020	-2.83	0.0061	.56

7. Appendix 2: Plate lysimeter calculations

Example calculation for N release from prunings in plate lysimeters, units are % of added N:

Table 7.1 Masses of material applied and total mass of N applied

	% N	% MC	FW. applied	DW applied	N grams
Inga pure	3.58	70.0	33.7	19.824	0.7097
CM pure	3.29	163.0	54.8	20.835	0.6855
Shaina	2.78	85.7	34.4	18.525	0.5150
Inga mix	3.63	69.7	16.9	9.959	0.3615
CM mix	3.13	73.3	27.4	15.815	0.4950
					0.8565

Step 1) Calculate the amount of mineral N in sample attributable to release by prunings (N concentration is from analysis by colorimetry, and is net (treatment minus controls):

= mg-N/l-water * l-water/plate

Centrosema (rep.6, day 47) = 2.87 mg/l * [(2.27 cm rain * 314.2 cm^2/plate)(1g/cm^3)/(1000g/l)]

= 2.87 mg/l * 0.713 l/plate

= 2.05 mg/plate

Step 2) Relate mg N sampled from plate to the total N applied to the plate:

% = (mg - N sampled / mg - N applied) * 100

Centrosema (rep.6, day 47) = (2.05 mg / 495)

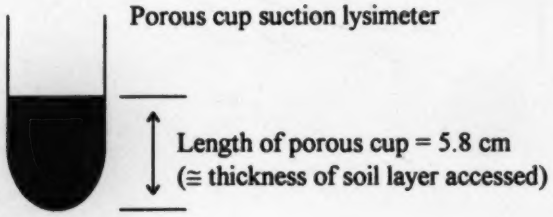
= 0.41

8. Appendix 3: Suction lysimeter calculations

Example calculation for % of N added in prunings sampled by suction lysimeters:

Step 1) Find soil water content available to lysimeter tension
= Volumetric water content (saturation) - Volumetric water content (wilting point)
= 40.8 % - 9.73 % (*means, 10-20 cm layer*)
= 31.07 %

Step 2) Find volume of a soil layer equivalent in thickness to the length of the porous cup and one hectare in area. For example:



= cup length * m^2/hectare
= 5.8 cm * 10 000 m^2/ha
= 580 m^3/ha
= 580 000 litres/ha

Step 3) Determine the volume of water in this soil volume.
= Volume of soil (from step 2) * Volumetric soil water content (from step 1)
= 580 000 l/ha * 0.3107
= 180206 l/ha

Step 4) Determine the amount (kg) of N sampled per hectare
= Volume of soil water (l/ha) * Concentration of mineral N in soil water (mg/l)
= 180206 l/ha * 70 mg/l (*Inga treatment, rep.1, day 13*)
= 12614420 mg/ha
= 12.61 kg/ha

Step 5) Determine the percentage of pruning N sampled by lysimeters
= [mineral N (treatment) - mineral N (control)] / pruning N added
= [12.61 kg/ha (*Inga treatment*) - 3.94 kg/ha (*Inga control*)] / 678.1 kg/ha (*Inga*)
= 1.28 %

9. Appendix 4: Maximum infiltration calculation

Example calculation for maximum infiltration of precipitation. Assumes that net drainage is equal to precipitation, which requires that soil is always at field capacity and zero evapotranspiration. There was evapotranspiration, so these estimates represent mazimums under the piston displacement model of soil water transfer.

= (Cumulative rain) * (1 / Volumetric water content at field capacity)
= 81.6 mm (11 days following burn) * 1 / 0.28 (vol. field cap. in 0-30 cm layer)
= 291.4 mm

10. Appendix 5: Selected Photos

Photo #1: Improved Fallow Vegetation

Photo #2: fine loamy, mixed, isohypothermic Typic Paleudult

Photo #3: Sub-plot in construction

Photo #4: Sub-plot (Inga prunings) with plate lysimeters and suction lysimeter

Photo #5: Slash and Burn Disturbance

Photo #6: Site following Slash and Burn

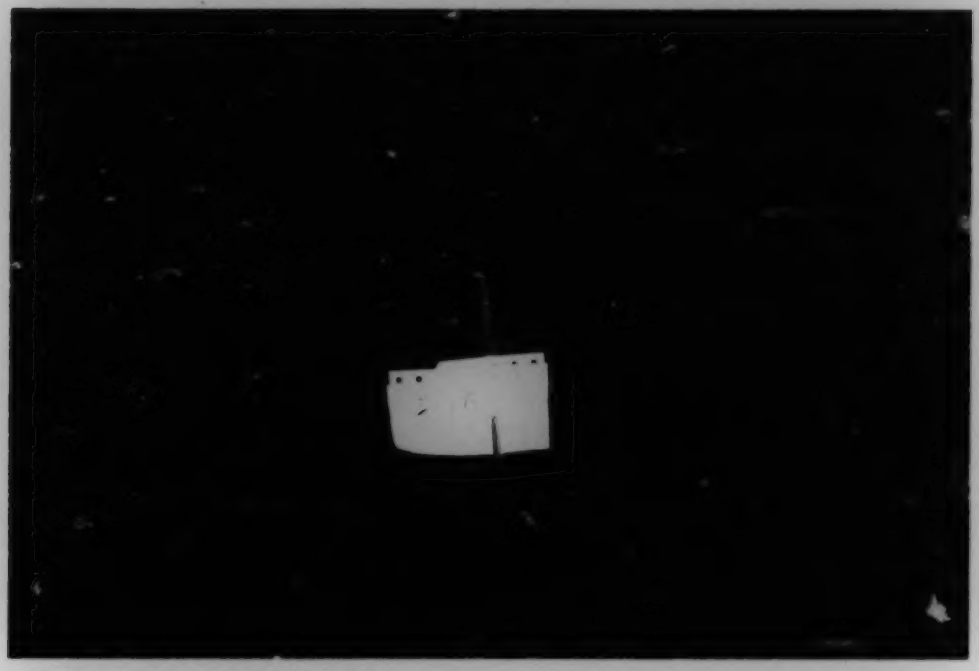


Photo #7: Ash in *Inga* fallow plot

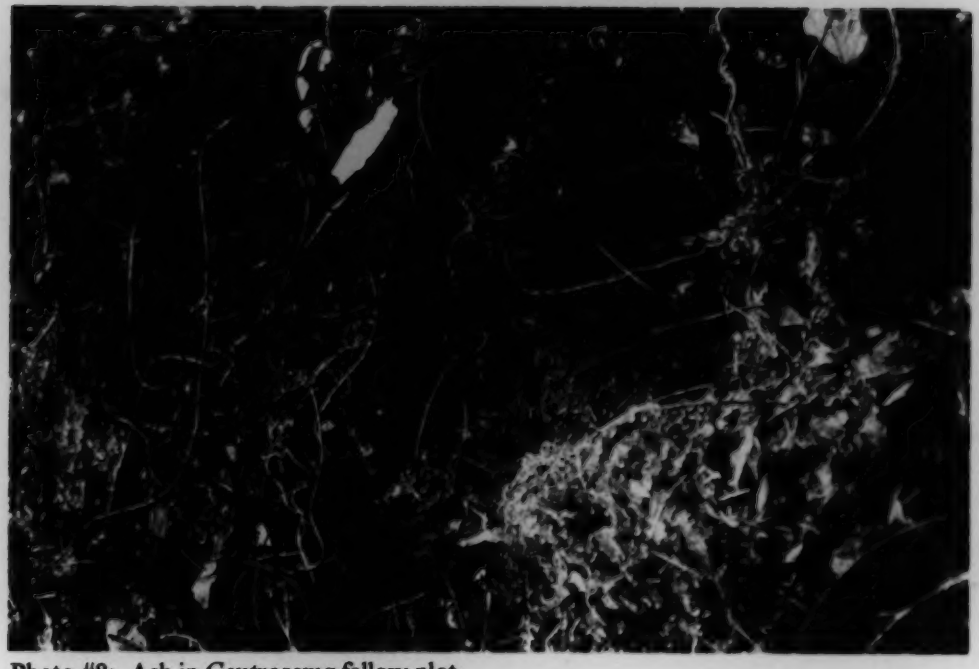


Photo #8: Ash in *Centrosema* fallow plot

Photo #9: Maize planting

Photo #10: Maize crop at ≅ 5 weeks

Photo #11: Column-incubation design

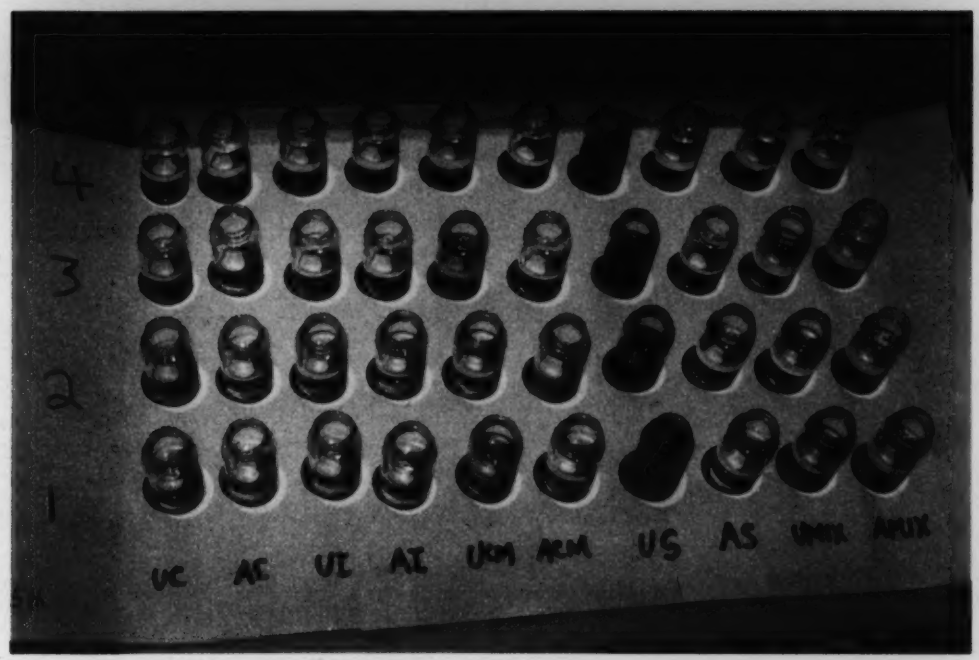


Photo #12: Column leachates at day 35

Photo #13: Preferential flow through a channel in the Yurimaguas Ultisol

Photo #14: Remaining organic matter on a *Colubrina*-Ultisol replicate.

11. Appendix 6: Personal impressions about some constraints to development in the Yurimaguas area, Peru

During my 6 months of work in Peru I was fortunate to work at the San Ramon Experiment Station and to live in Yurimaguas. At the experiment station, I had daily contact with station investigators, laboratory technicians and field workers. In Yurimaguas, I had contact with a range of local citizens including undergraduate students, young professionals, merchants, and neighbors. To the extent that my Spanish language skills allowed, I was able to discuss with my contacts some aspects of the history and current development status of the region. I was left with a some strong impression about Yurimaguas' past and the current regional constraints to development. I also formed some opinions about the requirements for development in the Yurimaguas area; these were not part of my formal research, I have not reviewed relevant literature, and I do not claim to have expertise in such assessments.

Jesuit priests were active in Yurimaguas well over (I believe) one hundred years ago. Rubber production was important in the early economy of the region, but when rubber began to be produced in other parts of the humid tropics production in this region died out. During this early era Yurimaguas was accessed by boat along the rivers which bound it on three sides.

More recently, the development of the Yurimaguas region was influenced by road access and an influx of in-migrants from the Peruvian highlands. The immigration is driven by land scarcity in the highlands and relative land abundance in the humid forests of the Amazon basin. Agricultural production in the lands surrounding Yurimaguas has been dominated by shifting cultivation, not unlike many other parts of the tropics. Two features of shifting cultivation in this area were striking for me. First, the large area which had been put into shifting cultivation in the past; it is not easy to find primary forest in the area anymore. Second, the vigorous re-growth of secondary forest after abandonment of agricultural land. I think that reports of the destruction of the Amazon forests which reach the Canadian public are misleading in this regard. The humid forests are being fragmented, and their age structure may be changing, but they are not necessarily being irrecoverably lost.

Perhaps because I have a background in Forestry in Canada, the secondary forests around Yurimaguas appeared unbelievably vigorous to me. I saw 25 year old, unmanaged forest stands near Yurimaguas which had the structure of a 150 year old forest stand in British Columbia or Alberta. I realized that although the Yurimaguas climate offers high photosynthetic potential, and the region contains very productive forest land, it is not an easy environment for people to grow cereal crops. Cereal crop production in this area is probably constrained most by the chemical characteristics of the soils and by aggressive

weeds and other pests. I was left with the paradoxical impression of a very rich ecosystem where people struggled to improve their welfare.

In my discussions with members of the community I learned that many people were very concerned about environmental degradation in their region. In 1997/1998 the El Nino Southern Oscillation phenomenon was occurring and I listened to many people's worries about the potential of abnormal weather and the increasing frequency of El Nino. I also heard many people say that they thought the climate was changing, although these discussions may have been more a function of human nature than actual climate change. I heard much concern about logging of the forests in the area ("the extraction of wood"). A public forum about the logging industry was held in November 1997 and generated a great deal of community interest. On various occasions I was told that the loggers were equally as amoral as were the narcotics lords who had been prominent in the area during the 1980s.

Apparently timber companies pay taxes to the government and receive cutting permits to localized areas of valuable timber. Logs are hauled to Yurimaguas to be processed into lumber, and I was told that most of the lumber is transported to Lima (over the Andes) for sale. At a local mill, I witnessed some extremely large and valuable logs being processed into lumber. One of the reported problems with the current system of allocating cutting permits is the lack of logging practice regulations or their enforcement. Also, it is said that although companies pay a reforestation tax, reforestation efforts rarely occur.

In political discussions I heard concerns that the Amazon region had been historically exploited in the interest of coastal regions (ex. Lima) of Peru. The poor access road connecting Yurimaguas with the rest of Peru is one of the largest constraints facing Yurimaguas producers who wish to access distant markets. In the late 1980's terrorist movements became very popular and were disruptive in the Yurimaguas area; the San Ramon Experiment Station was threatened.

I believe that the Amazon region of Peru has the potential to support much further economic development regionally and for the rest of Peru. The Amazon region is large, contains high plant diversity and can support rapid forest growth. I think these characteristics make the region unique globally and should form the basis of future production systems in the region. Forest production can include products such as tree fruit, wood extracts, wood fibre, and tourism. The competitive advantage of humid tropical regions in wood products is increasingly being recognized; compared to the forests of the north they have much faster growth rates, are closer to world markets, and have lower labour costs.

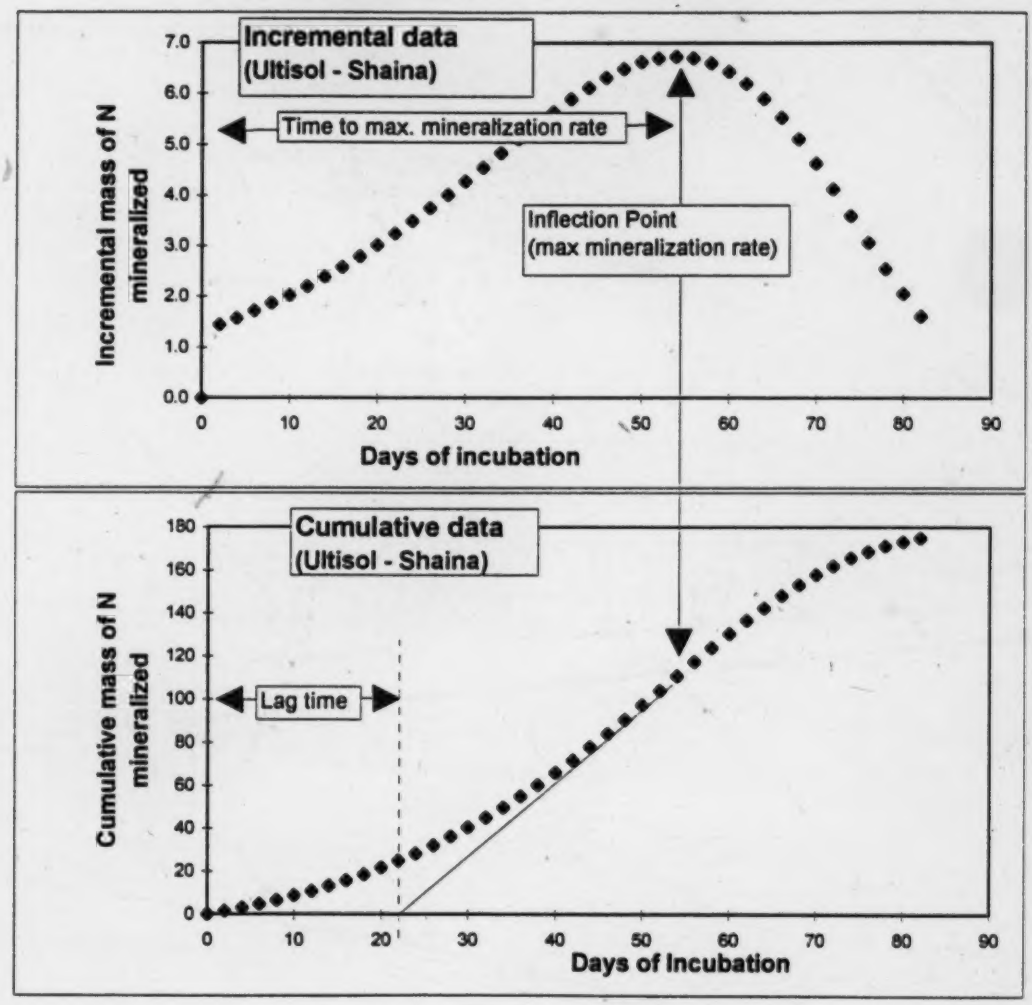
There are a number of basic requirements for the success of a forest sector in the Peruvian Amazon. First, a tenure system which encourages investment and sustainable production of forest products. Ultimately, I think that forest enterprises need long term tenure to limited areas of the Amazon forests. Second, I think that producers in the Amazon need

better marketing infrastructure connecting them to national and international markets. I think that marketing infrastructure must be built locally and gradually extended to higher economic levels. One of the problems with marketing Amazonian forest products will be adverse public opinion in industrialized nations and possibly resistance from forest producers in industrialized countries who will be threatened.

The development of a strong forest sector in Peru also requires favorable social and political conditions. Forest production requires long term investment which depends on political stability. Terrorist movements, although rooted in poverty and class struggle, are very disruptive. Forestry has the potential to employ large amounts of labour in plantation establishment, stand tending, harvesting, processing, marketing and sales, and service industries. A forest sector must bring these benefits to the residents of the region. This may help to improve the perception of forestry to the local public, and allow the social stability required for the region to thrive.

12. Appendix 7: Correlation coefficients

Correlation coefficients (r) between incremental % of pruning N released (PLs) and chemical characteristics of prunings. Note very little difference between coefficients for L:N, PP:N, and L+PP:N.

Sample Day	3	11	18	28	41	47
L+PP:N	-0.90	-0.80	-0.83	-0.60	-0.54	-0.77
C:N	-0.62	-0.62	-0.57	-0.45	-0.28	-0.69
L:N	-0.90	-0.80	-0.83	-0.60	-0.53	-0.76
PP:N	-0.85	-0.80	-0.80	-0.66	-0.58	-0.78

13. Appendix 8: Gompertz model variables in Ultisol-Shaina cumulative and incremental data.